IMMUNE SERA

A CONCISE EXPOSITION OF OUR PRESENT KNOWLEDGE CONCERNING THE CONSTITUTION AND MODE OF ACTION OF

ANTITOXINS, AGGLUTININS, HÆMOLYSINS, BACTERIOLYSINS, PRECIPITINS, CYTOTOXINS, AND OPSONINS

BY

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THIRD EDITION, ENLARGED

FIRST THOUSAND

NEW YORK

JOHN WILEY & SONS

LONDON; CHAPMAN & HALL, LIMITED

1908

The Scientific Press
Robert Drummond and Company
New York

PREFACE

THIS book has its origin in a monograph by Professor Wassermann, a translation of which was published by the author in 1904 under the title "Immune Sera." While much of the material contained in that book will be found in the present volume, it has been deemed necessary to discuss more fully the original topics, and to widen the scope of the book by adding chapters on snake venoms and their antisera, agglutinins, opsonins, and serum sickness. The author gratefully acknowledges his indebtedness to Dr. W. H. Park for valuable suggestions in the preparation of the book.

NEW YORK, *Sept.* 1, 1907.

NOTE TO THIRD EDITION

IN response to a number of inquiries, the author has added a chapter dealing with the serum diagnosis of syphilis. This concerns mainly the test devised by Wassermann and subsequently modified by various authors, though a brief description of the Porges-Meier test is also included. Through the courtesy of Dr. Noguchi, the author is also able to present a note on a simple, as yet unpublished, test devised by that investigator.

NEW YORK, *July* 15, 1908.

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ANTITOXINS

Historical. — The researches of Buchner[1] in 1889 had shown that the serum of animals artificially immunized against a certain bacterium possessed marked bactericidal properties for that particular organism. In studying immunity on animals which had been successfully immunized against diphtheria infection, Behring,[2] working in Koch's laboratory was struck by the fact that in these animals living virulent diphtheria bacilli were often demonstrable in the scab at the site of injection several weeks after the infection, and furthermore that the blood serum of the animals did not possess bactericidal properties. In a study published in 1890 Behring showed that the serum of rabbits artificially immunized against diphtheria was able to confer a specific immunity against diphtheria infections in other animals. He also demonstrated that such a serum could be used therapeutically to cure an infection already in progress. Such a serum

[1] Buchner, Centralblatt Bacteriologie, Vol. v. 1889. Archiv. f. Hygiene, Vol. x. 1890.

[2] Behring & Kitasato, Deutsche med. Wochenschrift, No. 49. 1890.

was not bactericidal, and retained its therapeutic power for a considerable time. He believed that the action of the serum was effected by a neutralization of the bacterial toxin by an "antitoxic serum constituent." The action was strictly specific, an antitoxic serum obtained after a diphtheria infection protected only against diphtheria; one derived from a tetanus animal, only against tetanus. Subsequently Behring and Knorr showed that immunization could be effected with bacterial-free filtrates of tetanus cultures and that the serum thus produced protected not only against tetanus infection but against poisoning by the toxic products of the bacilli. After considerable experimental work Behring and his collaborators devised an effective method of immunizing sheep and certain other animals against diphtheria and against tetanus and so produced antitoxic sera in considerable amounts.

The following account taken from Park shows the present methods of producing diphtheria antitoxin.

Production of the Diphtheria Toxin. — A strong diphtheria toxin should be obtained by taking a very virulent culture and growing it in broth which is about 8 cc. normal soda solution per liter above the neutral point to litmus. The culture fluid should be in comparatively thin layers and in large-necked Erlenmeyer flasks, so as to allow of a free access of air; the temperature should be about 35° to 36° C. The culture, after a weeks growth, is removed from the incubator,

and having been tested for purity by microscopic and culture tests is rendered sterile by the addition of 10 per cent of a 5 per cent solution of carbolic acid. After 48 hours the dead bacilli have settled on the bottom of the jar and the clear fluid is filtered through ordinary sterile filter paper and stored in full bottles in a cold place until needed. Its strength is then tested by giving a series of guinea pigs carefully measured amounts. Less than 0.01 cc. when injected hypodermically should kill a 250 gram guinea pig.

Immunizing the Animals. —The horses used should be young, vigorous, of fair size, and absolutely healthy. Vicious habits, such as kicking, etc., make no difference, except, of course, to those who handle the animals. The horses are severally injected with an amount of toxin sufficient to kill five thousand guinea pigs of 250 grams weight (about 20 cc. of strong toxin). After from three to five days, so soon as the fever reaction has subsided, a second subcutaneous injection of a slightly larger dose is given. With the first three injections of toxin 10,000 units of antitoxin are given. If antitoxin is not mixed with the first doses of toxin only one-tenth of the doses advised is to be given. At intervals of from five to eight days increasing injections of pure toxin are made until at the end of two months from ten to twenty times the original amount is given. There is absolutely no way of judging which horses will produce the highest grades of antitoxin. Very roughly those horses which are extremely sensitive, and those which react hardly at all are the poorest, but even here there are exceptions. The only way, therefore, is at the end of six weeks or two months to bleed the horses and test their serum. If only high grade serum is wanted all the horses that give less

than 150 units per cc. are discarded. If moderate grades only are desired, all that yield 100 units may be retained. The retained horses receive steadily increasing doses, the rapidity of the increase and the interval of time between the doses (three days to one week) depending somewhat on the reaction following the injection, an elevation of temperature of more than 3° F. being undesirable. At the end of three months the antitoxic serum of all the horses should contain over 300 units and in about 10 per cent as much as 800 units per cc. Very few horses ever give over 1000 units, and none so far has given as much as 2000 units per cc. The very best horses, if pushed to their limit continue to furnish blood of gradually decreasing strength. If every nine months an interval of three months' freedom from inoculations is given, the best horses furnish high grade serum during their periods of treatment for from two to four years.

Collecting the Serum. — In order to obtain the serum the blood is withdrawn from the jugular vein by means of a sharp-pointed canula which is plunged through the vein wall, a slit having been made in the skin. The blood is carried by a sterile rubber tube attached to the canula, into large Erlenmeyer flasks and allowed to clot, the flasks, however being placed in a slanting position before clotting has commenced. The serum is drawn off after 4 days by means of sterile glass and rubber tubing, and is stored in large flasks in a refrigerator. From this as needed small vials are filled. The vials and their stoppers, as indeed all the utensils used for holding the serum, must be absolutely sterile and every possible precaution must be taken to avoid contamination of the serum. An antiseptic may be added as a preservative, but is not necessary. Diph-

theria antitoxin, when stored in vials and kept in a cool place away from light and air contains within 10 per cent of its original strength for at least two months; after that it can be used by allowing for a maximum deterioration of 3 per cent for each month.

Testing the Strength of the Antitoxin. — This is carried out as follows: Six guinea pigs are injected with mixtures of toxin and antitoxin. In each of the mixtures there is 100 times the amount of a toxin (similar to that adopted as the standard) which will kill a 250 grams on an average in 96 hours. In each of the mixtures the amount of antitoxin varies; for instance, No. 1 would contain 0.002 cc. serum; No. 2, 0.003 cc.; No. 3, 0.004 cc.; No. 4, 0.005 cc., etc. If at the end of the fourth day Nos. 1, 2 and 3 were dead and Nos. 4, 5 and 6 were alive we would consider the serum to contain 200 units of antitoxin for each cubic centimeter. When we mix only ten fatal doses of toxin with one-tenth of the amount of antitoxin used with 100 fatal doses, the guinea pig must remain well. The mixed toxin and antitoxin must remain together for fifteen minutes before injecting.

Behring's publication was followed in the next two years by considerable work along these lines, valuable contributions being made by Aronson,[1] Roux, and Martin,[2] Wernicke,[3] Knorr[4] and others. The statements of Behring as to the strict specificity of the antitoxins were fully confirmed. Certain

[1] Berliner med. Gesellschaft, Sitzung, Dec. 21, 1892. Also Berliner Klin. Wochenschrift, 1893 and 1894.

[2] Roux and Martin, Annal. Pasteur 1894.

[3] Behring and Wernicke, Zeitsch. Hygiene, 1892. Vol. xi.

[4] Behring and Knorr, Zeitsch. f. Hygiene, 1893. Vol. xii.

observations by Buchner[1] and by Roux and Martin threw doubt, however, on the correctness of Behrings view that the toxin was neutralized by the specific serum just as a base was neutralized by an acid. It was claimed, for example, that the specific serum acted mainly on the body cells causing them to become non-susceptible to the poison in question. Various theories were formulated to account for the production of the antitoxins, their specificity, etc., but of them all only one has at all maintained itself. This, is the so-called side-chain theory, which was formulated by Ehrlich[2] in 1897.

Ehrlich's Side-Chain Theory. — Originally the side-chain theory was applied by Ehrlich only to the production of the specific *antitoxins*, i.e., substances in the blood, which act not only on the living bacteria, but also and especially on their dissolved toxins. Later on he extended it so as to apply also to the formation of specific bactericidal and hæmolytic substances in the serum of animals treated with living bacteria or with animal cells.

Toxins — Toxoids — Special Function of the Side Chains. — The basis of the theory is the fact that poison and counter-poison, toxin and antitoxin, combine directly in any given quantity. This combination always occurs in definite proportions

[1] Buchner, Münchener med. Wochenschrift, 1894.
[2] Ehrlich, Klinisches Jahrbuch, 1897.

following the laws of chemical combination; and, still following those laws, is slower at lower temperatures than at higher, stronger in concentrated than in dilute form. Ehrlich could further show that each poison for which by the process of immunizing one can develop a counter-poison possesses two groups which are concerned in the combination with the counter-poison or antitoxin. One of these, the so-called *haptophore group*, is the combining group proper; the other, the *toxophore group*, *is* the carrier of the poison. A poison molecule, therefore, might lose the one, the toxophore, and still be capable by means of its haptophore group of combining with antitoxin. Such a modified poison, which because of the loss of the toxophore group can hardly be called a poison, but which still possesses the power to combine with antitoxin, Ehrlich calls a *toxoid*. Toxoids may be produced spontaneously in old poisons through decomposition of the poison molecule, or they may be produced artificially by causing certain destructive agents such as heat or chemicals to act on bacterial poisons. The toxophore group is a very delicate one and much more readily decomposed than the combining (haptophore) group. Ehrlich reasoned that in order for a poison to be toxic to an organism, i.e., in order that the toxophore group be able to act destructively on a cell, it is necessary for the haptophore group of the poison to combine with

the cell. "In every living cell," Ehrlich says, "there must exist a dominating body [Leistungs Kern] and a number of other chemical groups or side chains. These groups have the greatest variety of function, but especially those of nutrition and assimilation."

The side chains, then, according to this author,

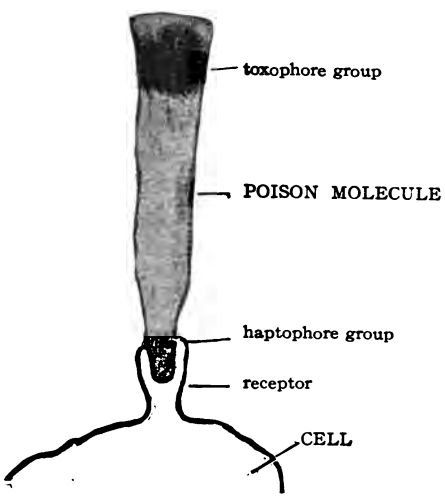


FIG. 1

are able to combine with the greatest variety of foreign substances and convert these into nourishment suitable to the requirements of the active central body. They are comparable to the pseudopodia of the lower animals, which engulf food particles and assimilate the same for the immediate use of the organism. In order that any substance

may combine with these side chains it is necessary that certain very definite relations exist between the combining group of the substance and that of the side chain. Using the well-known simile of Emil Fischer, the relation must be like that of lock and key, i.e., the two groups must fit accurately. Hence not every substance will fit all the side chains of an organism. It will combine only with those for which is possesses a fitting group.

Receptors — Weigert's Overproduction Theory. — This doctrine of the chemistry of the organism's metabolism Ehrlich applied to the action of toxins and antitoxins. "The toxin," he said, "can act only when its haptophore group happens to fit to one of the side chains," or *receptors*, as he now prefers to call them. As a result of this combination, the toxophore group is able to act on the cell and injure it. If we take as an example tetanus, in which all the symptoms are due to the central nervous system, the side-chain theory assumes that the haptophore group of the tetanus poison fits exactly and is combined with the side chain or receptors of the central nervous system. Other experiments, which we will not reproduce here, have shown us unquestionably that the action of the antitoxins depends on the fact that this combines with the haptophore group of the poison and so satisfies the latter's affinity. Ehrlich, therefore, concluded that the antitoxin is nothing else than

the side chains or receptors which are given off by the cells and thrust into the circulation. The way in which these side chains or receptors are thrust off as a result of the immunizing process, Ehrlich explains by means of *Weigert's Overproduction Theory.*

At the meeting of German Naturalists and Physicians held at Frankfurt in 1896, Weigert [1] in discussing regeneration, advanced an hypothesis the essential features of which are that physiological structure and function depend upon the equilibrium of the tissues maintained by virtue of mutual restraint between their component cells; that destruction of a single integer or group of integers of a tissue or a cell removes a corresponding amount of restraint at the point injured, and therefore destroys equilibrium and permits of the abnormal exhibition of bioplastic energies on the part of the remaining uninjured components, which activity may be viewed as a compensating hyperplasia; that hyperplasia is not, therefore, the *direct* result of external irritation, and cannot be, since the action of the irritant is destructive and is confined to the cells or integers of cells that it destroys, but occurs rather indirectly as a function of the surrounding uninjured tissues that have been excited to bioplastic activity through the removal of the restraint

[1] Weigert, Verhandlungen der Ges. deutscher Naturforscher und Aerzte, 1896.

hitherto exerted by the cells destroyed by the irritant; and, finally, when such bioplastic activity is called into play there is always hypercompensation — i.e. there is more plastic material generated than is necessary to compensate for the loss.

Ehrlich points out that owing to the combination of the toxin with the side chain of a cell, these side chains are practically lost to the cell; that the latter or its fellows now produces new side chains to replace this loss, but that this production always goes so far as to make a surplus of side chains; that these side chains are thrown off by the cell as unnecessary ballast, and then circulate in the blood as antitoxin. The same substances, therefore, which when part of the cell combine with the haptophore group of the toxin, enabling that to act on the cell, when circulating free in the blood combine with and satisfy this haptophore group of the toxin, and prevent the poison from combining with and damaging the cells of the organism.

It does not follow from Ehrlich's theory that the antitoxin is produced by the same set of cells whose injury by the toxin gives rise to the particular clinical symptoms. Thus we might believe that although in tetanus the cells of the central nervous system give rise to the characteristic symptoms, cells entirely apart from these, e.g., in the bone marrow, might be the main source of the antitoxin. The

fact that we appreciate symptoms from only one organ is, obviously, no proof that other tissues have been unaffected.

It may be well here to call attention to another rather common misconception regarding the production of antitoxin, namely that the body cells have to become educated, so to speak, to produce the antitoxin. This, it is believed, is effected by giving gradually increasing doses of toxin. As a matter of fact the reason for this gradual increase in the dose injected is quite different. The object in view is the administration of an enormously large dose of toxin, one that will engage the receptors of many cells. The previous injections have brought about some production of antitoxin and this partially neurtalizes some of the toxin injected, making it possible to give a larger dose than before. If one gives at the outset a large amount of toxin, partially neutralized by antitoxin, one will produce an amount of antitoxin equal to that ordinarily obtained in response to the same quantity of unaltered toxin given as the tenth or twentieth injection of a series. Park and Atkinson for example, injected a fresh horse with one litre of a toxin neutralized 1½ times for guinea pigs. At the end of a week the horse had produced a serum containing 60 units per cc. When the toxin was neutralized 6 fold no antitoxin whatever was produced.

Experimental Evidence for Ehrlich's Theory. — According to Ehrlich, then, the formation of specific antibodies must proceed in three stages:

1. The binding of the haptophore group to the receptor.

2. The increased production of the receptors following this binding.

3. The thrusting-off of these increased receptors into the blood.

So far as the first point is concerned Wassermann [1] showed that with tetanus, in which, as is well known, all the symptoms are referable to the central nervous system, tetanus toxin was bound by central nervous system substance in vitro. A mixture of tetanus poison and normal central nervous system was innocuous to animals, showing that certain substances present in the central nervous system combine with and thus satisfy the affinity of the haptophore group of the poison. This of course prevents the latter from combining with any cells of the organism. Organs other than the central nervous system do not possess this property of combining with tetanus poison, just as the central nervous system is, on the contrary, incapable of combining with diphtheria poison, which clinically does not show any pronounced affinity for the central nervous system.

Wassermann [2] also believes recently to have given

[1] Wassermann and Takaki, Berliner Klin. Wochenschr, 1898.

[2] Wassermann, New York Medical Journal, 1904.

experimental proof of the second and third points, the increased production of the receptors and their thrusting off. For this purpose he employed a tetanus poison which he had kept for about eight years, and which was originally very poisonous. In the course of years, however, owing to the damaging action of light, of oxidation, etc., it had become so weak that it was no longer toxic at all. Injections of one cc. into a guinea pig produced no tetanus. Nevertheless the haptophore group remained intact, as could readily be proved, for this non-poisonous tetanus toxin was still able to bind tetanus antitoxin, i.e. thrust-off receptors. On injecting rabbits with this non-poisonous tetanus toxoid in increasing doses, and then examining the blood serum of the animal he found not a trace of tetanus antitoxin. This absence could have either of two causes: It might be that the toxoid no longer produced any physiological effect whatever in the organism; or although it still caused an increase in the receptors, these increased receptors remained in the organs (sessile) and were not thrust off into the blood. In order to decide this question Wassermann first determined the exact quantity of fresh tetanus toxin which constituted a fatal dose for guinea pigs. He reasoned that if he injected first the toxoid, and shortly after, say in one or two hours, the fresh toxin, he should in such an animal have to increase the fatal dose,

i.e. more tetanus toxin should be required to kill this animal than a normal one, because owing to the previous toxoid injection part of the cells susceptible to tetanus toxin would already have been occupied. Provided Ehrlich's theory were correct, so that this binding of the toxoid really occurred, the conditions should be entirely different when, instead of injecting the toxin shortly after the toxoid, he waited somewhat longer, one to three days, and then injected the fresh tetanus toxin. In that case Weigert's law should come into play and the receptors have commenced to increase in number, i.e. the organ should now possess more sensitive groups than before. This would manifest itself in such fashion that in contrast to the first experiment the fatal dose of fresh tetanus toxin could now be decreased; in other words a small dose would now tetanize the animal in a shorter time.

As a matter of fact Wassermann's experiments yielded exactly the results deduced theoretically. He injected a guinea pig with some of the non-poisonous toxoid and then, an hour later, with tetanus toxin. He found that much more toxin was required to kill this animal than a normal guinea pig of equal size. When, on the contrary, he waited one to three days, it was found that then a dose of tetanus toxin which would not even tetanize a normal guinea pig was sufficient to kill this one.

It will be seen that in the above experiments the completely non-poisonous toxoid, although it effected an increased production of receptors, did not cause their thrusting-off. The serum of the rabbit treated with toxoid contained no antitoxin whatever. Wassermann concludes from this and other experiments that the thrusting-off cannot be a function of the haptophore group, and that something additional is required. This "something," he claims is a function of the toxophore group. It may be stated that Von Dungern has also published experiments (with majaplasm) pointing to the existence of the second stage, the stage of sessile receptors.

Antigens or Haptins. — It has been found that it is impossible to produce any immunity against all poisons, e.g. strychnine or morphine. According to Ehrlich these simpler chemical molecules do not enter into a true chemical combination with the tissues, but form rather a kind of solid solution, a loose combination with the cells, so that they can again be abstracted from these cells by all kinds of solvents, e.g. by shaking out with ether or chloroform. The point can perhaps be likened to the difference between saccharin and sugar. Both substances taste sweet, but despite this similarity in their physiological action they behave very differently toward the cells of the organism. Saccharin simply passes through the organism without

entering into a firm combination, i.e. without being assimilated, and is therefore no food. Its sweetening action is a mere contact effect on the cells sensitive to taste. Sugar, on the contrary, is actually bound by the cells, assimilated and burnt, and so is a true food. Until recently it was believed that the simpler chemical substances could not excite the production of antibodies. Ford and Abel[1] have however been able to show that toad stool poison, a true toxin, against which an antitoxin can be produced is chemically a glucoside.

As we shall subsequently see it is possible to immunize the animal body against a large number of substances, including not only such cell products as ferments, toxins and venoms, but also cells of the greatest variety, bacteria, dissolved proteids, etc. All these substances, therefore, must possess haptophore groups able to combine with the side chains or receptors in the animal body. Collectively, we speak of such substances as *antigens* or *haptins*.

Nature of Antitoxins in General. — But little is known concerning the constitution of antitoxins, for we do not know them apart from serum or serum constituents. It seems probable that they are proteid in character, but this has not been positively decided. It has been found that like the globulins they are quite resistant to the action of trypsin, but are acted on by pepsin-hydrochloric

[1] Ford and Abel. Journal of Biological Chemistry, Vol. ii, 1907.

acid. In general they withstand a fair degree of heat, certainly far more than the toxins. Antitoxins are to be regarded as inactive substances, effecting merely a blocking of the haptophore group of the corresponding toxin. They do not act on the toxins destructively. This is indicated by experiments of Wassermann on pyocyaneus toxin, and of Calmette and Morgenroth [1] on snake venom, which showed that in the toxin-antitoxin combination, the toxin could again manifest itself after the antitoxin had been destroyed. The antitoxins therefore are not ferment-like substances. As far back as 1897 attempts were made to determine the chemical nature of the antitoxins. In that year Belfanti and Carbone [2] found that the antitoxin was precipitated with the globulins of the serum by means of magnesium sulphate. Dieudonné [3] had previously shown that the proteids thrown out of solution by acetic and carbonic acids contained none of the antitoxin. In 1901 Atkinson [4] showed that the globulins increase markedly in the serum of horses as the antitoxic strength increases. The most recent work on this subject is that of Gibson,[5] who shows that if the ammonium sulphate precipi-

[1] Morgenroth, Berlin. klin. Wochenschr. 1905.

[2] Belfanti and Carbone, Centralblatt Bacteriologie (Ref.), Vol. xxiii, 1898.

[3] Dieudonné, Arbeiten a.d. kaiserl. Gesundheitsamte. Vol. xiii, 1897.

[4] Atkinson, Jour. Exper. Medicine, Vol. i, 1901.

[5] Gibson, Journ. Biological Chemistry, Vol. 1, 1906.

tate (globulins, nucleo-proteids, etc.) is treated with saturated sodium chloride solution, practically all the antitoxic fraction passes into solution.

This author has recently studied the possibility of differentiating other antibodies by means of their precipitation characteristics. He believes that a differentiation of the antibodies into those precipitated with the pseudo globulins and with the euglobulin fractions, according to the Hofmeister classification, is based on a misconception of the application of ammonium sulphate in separating proteids by their precipitation characters. While there seem to be some differences in the distribution of the antibodies in individual specific sera in comparative experiments, this is not so absolute as maintained by Pick[1] and others. Gibson's work on the fractionating of poly agglutinative serum shows that no separation of the several antibodies developed in an individual serum is possible. In the case of antitoxic sera both Gibson and Ledingham find that in goat serum the antitoxin is not invariably associated with the euglobulin fraction as maintained by Pick, but shows the same solubilities as that in horse serum.

Toxins and other Poisonous Cell Derivatives, in General. — Soon after bacteriology had demonstrated the etiological connection between bacteria and disease, the conviction gained ground that it

[1] Pick, Beiträge z. chem. Physiol. u. Pathol., Vol. 1, 1901.

was less the actual destruction wrought by the bacteria directly, than the injury produced by their chemical products that gave rise to the lesions in the infectious diseases. Brieger, especially, was one of the first to direct attention to the probable existence of specific poisons in the bacteria. He isolated a number of well defined chemical substances called ptomaines, most of which were highly toxic. Subsequent study, however, showed that these were not the specific bacterial poisons. The latter, the true toxins are something quite different as we shall see in a moment. Still later other substances were isolated from bacteria, and these were termed toxalbumins. We now know that some of these were identical with the true toxins, but that others were entirely unrelated.

What then are the true toxins? A number of pathogenic bacteria, when grown in pure culture, produce *dissolved* poisons in the culture fluid. These poisons are neither ptomaines nor proteid substances; their chemical nature is still absolutely unknown. They are extremely sensitive to external influences, especially against heat, and in many ways are very analogous to ferments. Physiologically the toxins are extremely poisonous, far beyond that of any of the ordinary well known poisons, and this poisonous action manifests itself only after a certain latent period known as the *period of incubation*. Finally one of the funda-

mental properties of the toxins is their ability to excite, in the organism attacked, antitoxins directed specifically against them, so that for every true toxin there is a corresponding antitoxin.

In addition to these bacterial toxins we know of other poisonous substances possessing similar characteristics. Among these are the "zoötoxins," — snake venoms, spider and toad poisons, the toxin of eel blood, and the "phytotoxins," — ricin, crotin, abrin, etc. It may be mentioned that some of these are of somewhat more complex constitution than the ordinary bacterial toxins. Ricin, for example, appear to possess one haptophore group but two ergophore groups, a toxic and an agglutinating one. In the case of the snake venoms it is not yet definitely known whether they are haptins of the first order or of the second.

The Relations Existing between Toxin and Antitoxin. — The exact nature of the toxin-antitoxin reaction has long been the subject of study and has given rise to considerable discussion. For obvious reasons most of the work has been done with diphtheria and tetanus toxins and their antitoxins. In order to give the reader some conception of the diverging views of various authorities we shall devote a few pages to a brief study of the diphtheria toxin-antitoxin reaction.

During the earlier years of toxin-antitoxin investigations the filtered or sterilized bouillon, in

which the diphtheria bacillus had grown and produced its "toxin," was supposed to require for its neutralization an amount of antitoxin directly proportional to its toxicity as tested in guinea pigs. Thus, if from one bouillon culture ten fatal doses of "toxin" were required to neutralize a certain quantity of antitoxin, it was believed that ten fatal doses from every culture, without regard to the way in which it had been produced or preserved, would also neutralize the same amount of antitoxin. Upon this belief was founded the Behring-Ehrlich definition of an antitoxin unit.[1]

The results of tests by different experimenters of the same antitoxic serum, but with different diphtheria toxins, proved this opinion to be incorrect. Ehrlich[2] deserves the credit for first clearly perceiving and calling attention to this fact. He obtained from various sources twelve toxins and compared their neutralizing value upon antitoxin; these tests gave interesting and important information. The following table gives the results in four of his toxins and well illustrates the point in question:

[1] This unit was "ten times the amount of antitoxic serum necessary to just protect a 250 gramme guinea pig against ten fatal doses of the toxin."

[2] Ehrlich, Die Werthbemessung des Diphtherieheilserums. Klinisches Jahrbuch, 1897.

Serial Number.	Estimated minimal fatal dose for 250 gm. guinea pigs.	Smallest number of fatal doses of toxic bouillon required to kill a 250 gm. guinea pig within 5 days when mixed with one antitoxin unit. ("L_f Ehrlich.")	Fatal doses required to "completely neutralize" one antitoxin unit as determined by the health of the guinea pig remaining unaffected. ("L_0 Ehrlich.")	L_f minus L_0 in fatal doses.	Remarks.
A	0.009 cc.	39.4	33.4	6	Old; deteriorated from 0.003 to 0.009.
B	0.0165 cc.	76.3	54.4	22	Fresh toxin, preserved with tricresol.
C	0.039 cc.	123.	108.	15	A number of fresh cultures, grown at 37° C. four and eight days.
D	0.0025 cc.	100	50	50	Tested immediately after its withdrawal.

It was natural to suppose, as the early investigators did, that a just neutral mixture of toxin and antitoxin, would require the addition of but one fatal dose of toxin in order to regularly kill the test animal. In the above table, however, we see that this difference ranges from six to fifty fatal doses.

Partial Saturation Method — Toxons, Toxoids. — Ehrlich obtained considerable additional information by means of his "partial saturation" method. Certain experiments had led him to believe that the original antitoxin on which he had based his "unit" determinations, while able to neutralize 100 fatal doses (per unit) really represented 200 "binding

units," and that the toxic bouillon really contained several kinds of poisonous substances able to combine with antitoxin.

He now believes that the diphtheria bacilli excrete at least two such poisons, " toxins " and " toxons;" that these very quickly decompose to a greater or less extent forming various " toxoids."

In the case of a hypothetically pure toxin Ehrlich believes that one antitoxic unit would correspond to 200 fatal doses or 200 binding units. If the entire amount of antitoxin, i.e. $\frac{200}{200}$ is added to the amount of toxin in question, the result will be just complete neutralization. If the toxin is entirely pure, $\frac{199}{200}$ of the antitoxin unit would neutralize all but $\frac{1}{200}$ of the initial toxicity and $\frac{150}{200}$, or $\frac{100}{200}$ or $\frac{75}{200}$, etc. of the antitoxin added would permit corresponding degrees of toxicity to be demonstrated through animal inoculations. It was found, however, that neutralization according to this simple scale did not take place. The results were complicated and Ehrlich

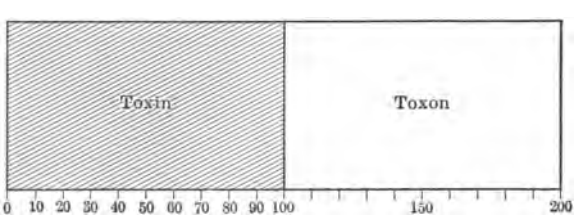


FIG. 2.

found it convenient to express them graphically in the form of the so-called " toxin spectra." Without

going much deeper into the subject the point may be illustrated by the appended diagrams or " spectra."

Fig. 2 shows the simplest conceivable diphtheria poison. In this case the following values would be obtained.

x^{cc} poison (100 fatal doses) + $\frac{999}{1000}$ antitoxin units = 0, i.e. absolutely neutral.

x^{cc} poison + $\frac{998}{1000}$ = Free toxon.

x^{cc} poison + $\frac{997}{1000}$ = Free toxon.

That is to say, if the proportion of antitoxin added was $\frac{998}{1000}$ of the amount required for complete neutralization, it would be found that the poison thus uncombined was much less, and *differently* toxic than a corresponding amount of the original toxin. It was found that these fractions possessed a rather constant though low degree of toxicity with characteristic action. This consisted in the production of some local œdema, followed by a long incubation period, and finally the development of cachexia and paralysis. Ehrlich believes that this action is due to a separate poison excreted by the diphtheria bacillus which he calls a *toxon*.

If we continue with the above poison we shall obtain these values:

x^{cc} poison + $\frac{999}{1000}$ = Toxin action (1 fatal dose).

x^{cc} poison + $\frac{970}{1000}$ = 30 fatal doses.

x^{cc} poison + $\frac{910}{1000}$ = 90 fatal doses, etc.

That is to say, if we add only $\frac{999}{1000}$ units antitoxin, i.e. $\frac{1}{1000}$ unit less than in the $\frac{998}{1000}$ mixture, we find

that one fatal dose is set free. This relation would exist right to the end. The fact that in this experiment the toxins are liberated after the toxons, shows that the toxons have less affinity for the antitoxin than have the toxins.

As a matter of fact, however, conditions are probably never as simple as this. In the process of toxin formation a double action is always going on — that of toxin and toxon production, and that of their decomposition. As was pointed out on a previous page the poisons quickly change into non-poisonous toxoids, and these substances are still able to bind antitoxin.

This is shown in the following " spectrum."

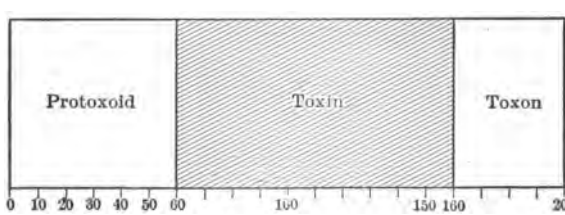


FIG. 3.

Here we would obtain the following figures:

x^{cc} poison + $\frac{200}{200}$ antitoxin unit = 0, i.e. absolutely neutral.

x^{cc} poison + $\frac{180}{200}$ = Toxon free.

x^{cc} poison + $\frac{160}{200}$ = Toxon free.

x^{cc} poison + $\frac{159}{200}$ = Toxin free (1 fatal dose.)

x^{cc} poison + $\frac{100}{200}$ = Toxin free (60 fatal doses.)

x^{cc} poison + $\frac{60}{200}$ = Toxin free (100 fatal doses.)

Now we come to the non-poisonous "prototoxoids":

$x^{cc} + \frac{59}{200}$ = Toxin free (100 fatal doses.)

$x^{cc} + \frac{30}{200}$ = Toxin free (100 fatal doses.)

$x^{cc} + \frac{1}{200}$ = Toxin free (100 fatal doses.)

We see here that after we have reduced the antitoxin to $\frac{60}{200}$ no further increase of toxicity is brought about by any further reductions. Ehrlich calls these toxoids "prototoxoids" because they have such a high affinity for the antitoxin. But there are apparently still other toxoids, as is shown by the following spectrum:

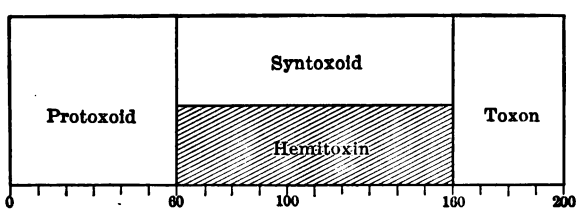


FIG. 4.

Here we would obtain values as follows:

x^{cc} poison + $\frac{200}{200}$ = 0, i.e. absolutely neutral.

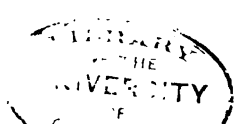
x^{cc} poison + $\frac{160}{200}$ = Toxon.

x^{cc} poison + $\frac{158}{200}$ = Toxin free (1 fatal dose).

x^{cc} poison + $\frac{156}{200}$ = Toxin free (2 fatal doses.)

x^{cc} poison + $\frac{100}{200}$ = Toxon free (30 fatal doses.)

Here we find that in the middle part of the "spectrum" we encounter a zone in which each $\frac{2}{200}$ antitoxin unit neutralizes one fatal dose. Ehrlich believes that this part of the mixture consists of



equal parts of syntoxoid and toxin — that is to say, he believes there are also toxoids which have the same degree of affinity for antitoxin that this toxic has. He speaks of these as "syntoxoids."

Views of Arrhenius, Bordet and Others. — Bordet and others refuse to accept Ehrlich's views and the whole matter is at the present time under active discussion. Thus the existence or non-existence of toxons has excited a great deal of discussion among investigators. The great Swedish chemist, Arrhenius, has recently given much attention to the toxins and has applied the principles of physical chemistry to the toxin-antitoxin reaction. It is, of course, well known that a solution of a compound such as sodium chloride represents not only NaCl in solution, but also sodium ions and chlorine ions. There is a certain amount of dissociation going on hand in hand with a combination of the two components. The degree of this varies with the temperature and the dilution of the substances. Arrhenius believes that the same process goes on with the toxin-antitoxin combination and that such more or less dissociated compounds give rise to the effects Ehrlich ascribes to the toxons.

Bordet has attempted to explain the toxon phenomena in a different way. He shows that the toxin molecule can combine with antitoxin in varying proportions. One would then assume that the toxin molecule possesses several "binding"

groups. The complete occupation of these groups causes the toxicity to be entirely lost, whereas partial saturation so affects the molecule that it exerts a milder and different action.

The principles of colloid chemistry have also been applied to the study of the toxin-antitoxin combination. Field[1] has recently tested the electrical charge of toxins and antitoxins and finds that both diphtheria and tetanus toxin and their antitoxins are electropositive, passing to the cathode pole. He concludes that the combination of toxin and antitoxin may perhaps represent not a true chemical reaction, but the absorption of one colloid by another. Ehrlich, however, still adheres to his views and points out that the advocates of colloid chemistry have been compelled to assume the existence of specific atomic groupings very much after his own ideas. He also cites van Calcar[2] who claims to have separated toxin and toxon by a dialyzing procedure.

[1] Field and Teague, Journ. of Exper. Medicine, Vol. ix, 1907.
[2] van Calcar, Berlin, Klin Wochenschr, 1905.

AGGLUTININS

The Agglutination Phenomenon. — We have just seen that pathogenic bacteria may be divided into those which produce extracellular toxins in culture media, and those which do not. Against the former the organism defends itself by the production of antitoxins; against the latter it produces a variety of antibodies: — bacteriolysins, agglutinins, precipitins, opsonins and possibly others.

The agglutinins can be observed either in a test-tube or in a microscopical preparation. For example, if typhoid or cholera immune sera are added respectively to a 24-hour culture of typhoid or cholera bacilli, and the mixture placed in a thermostat, the following phenomenon will be noticed: The bacteria which previously clouded the bouillon uniformly, clump together into little masses, settle to the sides of the test-tube and gradually fall to the bottom until the fluid is almost entirely clear. In a control test, on the contrary, to which no active serum is added, the fluid remains uniformly cloudy. The reaction is completed in twenty-four hours at the most. If the reaction is observed in a hanging drop, it is seen that the addition of the active serum first produces an increased motility of the

bacteria which lasts a short time and is followed by a gradual formation of clumps. One gets the impression that the bacteria are dying together. Frequently one sees bacteria which have recently joined a group make violent motions as though they were attempting to tear themselves away; then they gradually lose their motility completely. Even the larger groups of bacteria may exhibit movement as a whole. After not more than one or two hours the reaction is completed; in place of the bacteria moving quickly across the field, one sees one or several groups of absolutely immobile bacilli. Now and then in a number of preparations one sees a few separate bacteria still moving about among the groups. If the reaction is feeble, either because the immune serum has been strongly diluted or because it contains very little agglutinin, the groups are small and one finds comparatively many isolated and perhaps also moving bacteria. It is essential each time to make a control test of the same bacterial culture without the addition of serum. Under some circumstances the reaction proceeds with extraordinary rapidity so that the bacilli are clumped almost immediately. By the time the microscopical slide has been prepared and brought into view nothing is to be seen of any moving or isolated bacteria, and only by means of the control test is it possible to tell whether the culture possessed normal motility.

We are not yet informed as to the nature of these phenomena. A number of theories have been advanced, into which, however, we cannot here enter.

In some cases the agglutinins are active even in very high dilutions. Thus in typhoid patients and typhoid convalescents a distinct agglutination has been observed in dilutions of 1 : 5000, and this action persisted for years, though not, of course, in the same degree. Even normal blood-serum, when undiluted, often produces agglutination. But the above specific agglutinins, which do not exist beforehand, being formed only in consequence of an infection, are characterized by this, that the agglutination occurs even when the serum is diluted (at least 1 : 30 to 1 : 50), and, furthermore, that after this dilution the action is still specific, i.e. cholera immune serum agglutinates only cholera bacilli, typhoid immune serum only typhoid bacilli, etc. This specificity, however, as will be shown later, is not always absolute.

Agglutinins can also be developed against red blood cells and against certain protozoa (trypanosomes). We speak of the former as *hæmagglutinins*. Analogous to the hæmolytic action or *normal* serum on the red cells of certain other species, we find that *normal* serum is able to *agglutinate* the red cells of many species and bacteria. For example, normal goat serum agglutinates the red cells of man, pigeon, and rabbit;

normal rabbit serum agglutinates typhoid and cholera bacilli.

Purpose of Agglutination. — It is not yet clear what the purpose, if any, of the agglutinating function is. Gruber, the first to thoroughly study and appreciate the bacterial agglutinins, assumes that the process injures the affected cell, preparing it for solution and destruction. After numerous experiments I have not been able to convince myself of any damaging influence of the agglutinins on the affected cell, be this blood cell or bacterium, and the observations of other authors confirm this opinion. Agglutinated bacteria are capable of living and of reproduction, and agglutinated red blood cells are no more fragile or easier to destroy than normal, non-agglutinated cells. Neither can anything be discovered microscopically which would indicate any injury to their structure.

One thing is certain: that the agglutinins are in no way related to the lysins found in serum, and so of course are not identical with these. The simultaneous occurrence in a serum of immune bodies, interbodies, complements, and agglutinins is an entirely independent phenomenon which is in no way regular. There are sera which dissolve certain cells without agglutinating them, and others which agglutinate cells without dissolving them.

Historical. — Serum diagnosis by means of the

agglutinins was introduced chiefly through the labors of Gruber and Widal. The studies undertaken by Gruber and his pupil Durham began as early as 1894. At the Congress for Internal Medicine in 1896 [1] Gruber first announced that he had discovered the reaction in typhoid convalescents, and asked that his observations be verified if possible. Soon after this Pfeiffer and his co-workers published a study which confirmed Gruber's results.[2] The significance of the reaction as a diagnostic help was unquestionably first pointed out by Widal,[3] who showed that the reaction appears at a relatively early period of the disease, and may therefore be employed as a diagnostic measure. We must not omit to state that Grünbaum[4] in March, 1896, several months before Widal's publication, had also grasped the significance of the reaction as a diagnostic measure. Owing to insufficient clinical material his publication did not appear until some time after Widal's. Hence, in acknowledgment of the labors of the two authors most concerned in the discovery and introduction of this reaction, we now speak of it as the "Gruber-Widal reaction," whereas in

[1] Transactions of the Congress, edited by E. von Leyden and R. Pfeiffer, Wiesbaden, 1896.

[2] Pfeiffer and Kolle, Deutsche med. Wochenschrift, 1896, No. 12.

[3] Widal, Bulletin de la soc. méd. des hôp., June 26, 1896.

[4] Grünbaum, Lancet, Sept. 19, 1896; Muench. med. Wochenschrift, 1897, No. 13; Blood and the identification of bacterial species, Science Progress, Vol. I, No. 5, 1897.

the beginning only the term "Widal reaction" was used.

The manner in which the reaction proceeds in microscopical preparations as well as when macroscopically observed has been described above (page 30). Nowadays the microscopic method is given the preference [1] because in many cases it is distinct when the macroscopic reaction fails; and further because the former yields distinct results within an hour at the most, whereas in many cases twenty-four hours are required for the macroscopic test.

Pfaundler's Reaction (Thread Reaction). — It may be well at this point to call attention to a peculiar reaction described by Pfaundler [2] in 1896. This author showed that certain bacteria, though they might not be agglutinated by a given serum, would often, when they were grown therein, develop in the form of long threads more or less interlaced. This occurred only in the specific serum and was absent in the normal serum. Most authorities regard the thread reaction as a manifestation of agglutinins. According to Metchnikoff this reaction sometimes gives more information concerning a serum than does the ordinary agglutination test.

Nature of the Agglutinins. — The agglutinins are

[1] This applies to typhoid; in other diseases the macroscopic method is sometimes preferable.

[2] Pfaundler, Centralblatt Bacteriologie, Vol. xix, 1896.

fairly resistant substances which withstand heating to 60° C., and lose their power only on heating to 65° C. It is possible, therefore, to make a serum bacteriolytically inactive by heating to 55° C., and still preserve its agglutinating power. Corresponding to the specific combining power of these agglutinins, they possess a haptophore group which effects the combination, and a second group, easily decomposed by acids, which effects the clumping. In the bacterium as well as in the blood cell there exists a substance not yet closely studied, called the *agglutinable substance*. This also has two groups, a haptophore, which combines with the haptophore group of the agglutinin; and a second, more delicate group, which is acted on by the functional group of the agglutinin.

Nature of the Agglutination Reaction. — The union of agglutinin with the agglutinable substance is a chemical reaction, and is quantitative. The amount of bacteria in the emulsion used to test the amount of agglutinin must, therefore, be known. An emulsion one hundred times as dense as another would require one hundred times as much agglutinin to give an equally complete reaction. Agglutinin acts both on living and on dead bacteria.

The influence of salts upon agglutination is in a sense comparable to their action upon the precipitins. Joos found that antityphoid serum did not agglutinate typhoid bacilli in the absence of

salts. For agglutination to take place he considers it is as necessary as the agglutinin and agglutinable substance. He believes that salts play an *active* part in the process, a conception which is contrary to Bordet's, that the absence of salts offers only a physical impediment to agglutination. Friedberger does not consider that the salts act chemically for he found agglutination to take place in the presence of grape sugar, asparagin, etc.

In view of the fact that the protoplasm of the body and the albuminous constituents of serum have a close relationship to, or really are, colloids, investigators have studied certain reactions which occur among the colloids with the expectation that these would throw some light on the reactions of protoplasm and of serums.

Colloids diffuse very slowly and exert little or no osmotic pressure, supposedly because of the large size of the particles. They do not conduct electricity, but the particles react to the electric current by alterations in the direction of their motion (i.e. toward the positive or the negative pole), and, moreover carry electric charges themselves.

The features of colloids which bring them into relation with the subject in hand are their coagulable nature in certain instances and the fact that their particles may be agglutinated or precipitated by the addition of minute amounts of salts (electrolytes). This of course is entirely analogous

to the need of salts in the agglutination of bacteria by sera. In the latter reaction the agglutinins carry a positive, the bacteria a negative charge. The resulting combination, therefore, does not precipitate from the menstruum, supposedly because there is still sufficient difference in the electric potential. When salts are present the kations so alter the electric conditions of the colloidal particles, i.e., of the agglutinin-bacterium combination, that their surface tension is increased. In order to overcome this the particles get together, presenting in a clump less surface tension than if they remained as individual particles.

Agglutinoids. — Agglutinins which have lost their agglutinophore group through the action of acids, etc., but which still possess their haptophore group, are called *agglutinoids*, just as toxins which have lost their toxophore group are called toxoids. Such agglutinoids, then, may still combine with the blood cells or bacteria without being able, however, to produce any clumping or agglutination. The nature of agglutinoids, however, is still very obscure as is also the means by which they inhibit agglutination. It has occasionally been observed, for example, that agglutination is absent in concentrated serum, and present in dilute serum. This zone, of no agglutination preceding that of agglutination is often spoken of as the *pro zone*. It has been explained as due to the presence in the

serum of agglutinoids. These are assumed to possess a higher affinity for the bacteria than do the agglutinins and so prevent the latter from acting on the bacteria. Since, however, the agglutinins are usually far more abundant than the agglutinoids, dilution of the serum dilutes the latter to practically nothing, thus allowing the agglutinins, to combine with the bacteria. Some recent experiments by Field show that the *pro zone* may have an entirely different explanation, based on behavior of bacteria and agglutinin as colloids. It has already been stated that the union of agglutinin and bacterium does not precipitate because there is still sufficient electric potential; the combination carries a negative charge. Field believes that with very large amounts of agglutinin (as in the *pro zone*) the bacteria load themselves with so much agglutinin that the combination now carries a considerable positive charge. The surface tension therefore is not sufficient to cause a clumping to occur. Naturally the presence of salts does not alter the condition as the kations also carry a positive charge.

Group Agglutinins. — For some time after their discovery the agglutinins were regarded as strictly specific, i.e. a serum derived, for example, from a typhoid infection would agglutinate only typhoid bacilli and no others. After a time, however, it was found that such a serum would frequently aggluti-

nate somewhat related organisms, though not, usually, to so high a degree. In other words, while *agglutinins* may be nearly, if not quite, specific in their action, a serum which produces agglutination may be far from being so.

The following examples will illustrate the point. In a case of infection with paratyphoid bacilli, type B, the bacilli of the infecting type B were agglutinated 1 : 5700; typhoid bacilli, however, only 1 : 120, while paratyphoid bacilli type A were not agglutinated at all. In a case of typhoid infection an agglutination with a dilution of 1 : 40 was obtained for paratyphoid type B, while typhoid bacilli were agglutinated in a dilution of 1 : 300 and over. As a rule the agglutination with the infecting agent is by far the strongest, i.e. it proceeds even in high dilutions, whereas other bacteria require a stronger concentration.

In all this we are dealing with the same phenomenon which undoubtedly plays a rôle in the agglutination with blood of icteric patients, the so-called *group agglutination*, as it was first termed by Meinhard Pfaundler.[1] The bacteria which are agglutinated by one and the same serum need not at all be related in their morphological or other biological characteristics, as Pfaundler at first assumed. Conversely, micro-organisms which, because of the

[1] Über Gruppenagglutination und das Verhalten des Bacterium coli bei Typhus, Muench. med. Wochenschrift, 1899, No. 15.

characteristics mentioned, are regarded as entirely identical or almost so, are sharply differentiated by means of their agglutination. In other words, the " groups," arrived at by means of a common agglutination sometimes have no relation to species as the term is usually employed. Thus, according to Stern, certain varieties of proteus and of staphylococci excite the production of sera which exert marked agglutinating powers also on typhoid bacilli, although otherwise we do not regard these three microorganisms as at all related. On the other hand by means of agglutination we can sharply distinguish cholera bacilli from their nearest related species. Because of this lack of absolute specificity the *serum diagnosis of infection or the identification of bacteria* has value only when very carefully tested.

Absorption Methods for Differentiating between a Mixed and a Single Infection. — In 1902, Castellani[1] called attention to a procedure which consists in saturating the diluted immune serum with successive quantities of the bacteria most strongly agglutinated until the agglutinating power for these bacteria = 0. After centrifuging the mixture the clear fluid is tested on the second variety of bacteria, and from this one learns whether mixed or single infection was present. According to Castellani if the serum of an animal immunized against a certain microörganism is saturated with that organism,

[1] Castellani, Zeitschrift Hygiene, Vol. xl, 1902.

the serum will lose its agglutinating power not only for that organism but also for all other varieties that it formerly acted on. Saturated with the others, its action upon the first is reduced little or not at all.

The serum of an animal immunized against two microörganisms *A* and *B*, loses its agglutination when saturated with *A*, only for *A*. Saturated with *A* and *B*, it loses agglutinating power for both.

Park,[1] who has devoted considerable attention to this subject finds that the absorption method simply proves that when one variety of bacteria removes all agglutinins for a second, the agglutinins under question were not produced by that second variety.

Specific and group agglutinins may perhaps be better understood by means of the following diagram. We assume that the typhoid bacillus pos-

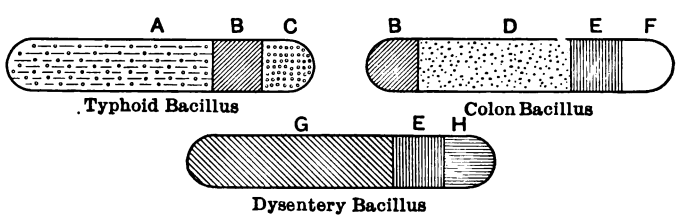


FIG. 5.

sesses considerable protoplasm *A*, which is specific for the typhoid bacillus, that it possesses also certain protoplasm *B*, which is common to it, and to the

[1] Park and Collins, Journ. Medical Research, Vol. vii, 1904.

colon bacillus, and some protoplasm *C*, common perhaps to some other bacterium. In the case of the colon bacillus, protoplasm *D* is specific, i.e., possessed only by this bacillus, while *B* is common to it and the typhoid bacillus, and *E* common to colon and dysentery bacilli.

By immunization with the typhoid bacillus we would obtain a serum containing agglutinins against protoplasm *A*, *B*, and *C*. By virtue of this the serum would exert some agglutinating power also on colon bacilli. On extracting such a serum with the typhoid bacilli, all the agglutinating power would be lost, that for the typhoid bacilli as well as that for the colon. On extracting this serum with the colon bacilli we would remove the agglutinating power for these bacilli, but leave the specific agglutinating power on typhoid bacilli.

Formation of the Agglutinins According to the Side-Chain Theory — Receptors of First, Second and Third Order. — Ehrlich's theory as outlined in the preceding chapter offers a ready explanation for the development of these bodies. Certain peculiarities of the agglutinins require merely a slight elaboration of detail in order to be clearly understood. According to Ehrlich the prime function of the side chains of a cell is to provide for the nutrition of the cell. Obviously the simplest mechanism for this purpose will be a side chain which merely anchors the food molecule, leaving the digestion entirely to

the cell proper. This type of receptor suffices for comparatively small molecules such as those of the toxins, for these are, after all, but the products of cellular activity. When the protoplasm of the bacterial cell itself, however, is to serve as food for the animal cell the latter needs more than a mere anchoring group, it needs also an active group which can in some way act on the huge food particle and make it more readily assimilable. Such receptors then possess two groups, a haptophore group and another functional group acting on the food particle thus anchored. Ehrlich calls these his " receptors of the second order," and places in this class the agglutinins and the precipitins. The same action can perhaps be more economically brought about by having these receptors, in addition to their specific haptophore group, possess the means by which the action of a ferment-like substance can be brought to bear on the anchored food particle. Such a receptor would then possess two haptophore groups, one for the food particle, the other for the ferment-like substance. These are Ehrlich's " receptors of the third order " and will be discussed in the next chapter. Confining ourselves for the present to the agglutinins we find that the existence of the two groups (haptophore and agglutinating) has experimental confirmation. We have seen that an agglutinin may be changed by the action, for instance, of acids, so that

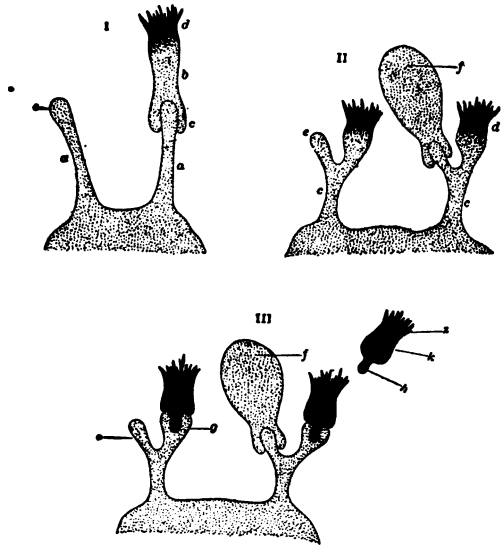


FIG. 6. — THE VARIOUS TYPES OF RECEPTORS ACCORDING TO EHRLICH.

I. *Receptors of the First Order.* — This type is pictured in *a*. The portion *e* represents the haptophore group, whilst *b* represents a toxin molecule, which possesses a haptophore group *c* and a toxophore group *d*. This represents the union of toxin and antitoxin, or ferment and antiferment, the union between antibody and the toxin or ferment being direct.

II. *Receptors of the Second Order* are pictured in *c*. Here *e* represents the haptophore group, and *d* the zymophore group of the receptor, *f* being the food molecule with which this receptor combines. Such receptors are possessed by agglutinins and precipitins. It is to be noted that the zymophore group is an integral part of the receptor.

III. *Receptors of the Third Order* are pictured in III, *e* being the haptophore group and *g* the complementophile group of the receptor. The complement *k* possesses a haptophore group *h* and zymotoxic group *s*; whilst *f* represents the food molecule which has become linked to the receptor. Such receptors are found in hæmolysins, bacteriolysins, and other cytolysins, the union with these cellular elements being effected by the amboceptor (a thrust-off receptor of this order). It is to be noted that the digesting body, the complement, is distinct from the receptor, a point in which these receptors therefore differ from those of the preceding order.

it will no longer possess any agglutinating action, but will still combine with the bacteria. In other words, the agglutinating group has been lost, the haptophore has remained intact. Once the agglutinating power is lost it cannot be restored, in which respect the agglutinins differ from the bacteriolysins.

BACTERIOLYSINS AND HÆMOLYSINS

Historical. — As far back as 1874, Gscheidlen and Traube [1] demonstrated that considerable quantities of septic material could be injected into the circulation of warm-blooded animals without apparently any effect on the animal. Very little was thought of this observation at the time, and it is not until more than ten years later that we find a similar observation made by Fodor.[2] In 1888 Nuttall [3] showed that normal blood serum possessed marked germicidal properties, and his observations stimulated a number of workers who undertook to determine the conditions most favorable to the exhibition of this phenomenon, and further to decide upon the constituent of the serum to which this property was due or whether it was a function of the serum as a whole. In 1899 Buchner [4] published a series of experiments and showed that an exposure of 55° C. robs the serum of its bactericidal property. He also concluded that the active element in the process was a living albumin and

[1] Gscheidlen and Traube. Schlez. Gesellschaft. f. Vaterland. Cultur, Med. Sect., 1874.

[2] Fodor, Deutsche med. Wochenschr, 1886.

[3] Nutall, Zeitschr. f. Hygiene, Vol. iv, 1888.

[4] Buchner, Centralblatt Bacteriologie, Vol. v, 1889. Archiv. f. Hygiene, Vol. x, 1890.

suggested for it the name " alexin." He found that it was possible to greatly increase the bactericidal action, (i.e. the quantity of " alexin ") for a particular bacterium by immunizing an animal with that bacterium.

Pfeiffer's Phenomenon. — An enormous advance in the study of immunity was made in the discovery of Pfeiffer's phenomenon in 1894, and it is to Pfeiffer's splendid observations [1] that we owe the first and most important insight into the mode of action of the bacteriolytic immune sera. A normal guinea pig is able to kill and dissolve a number of living cholera bacilli if these are injected intraperitoneally. If in such an animal we gradually increase the dose injected, it will be possible after a time to inject at one dose an amount of cholera bacilli that represents many times an ordinary fatal dose. If from this animal we now withdraw serum and inject it into another animal, we find that this serum, even in such small amounts as the fractional part of a centigram or even of a milligram, is able to protect the second animal against living cholera bacilli. Under the influence of these small amounts of serum of the treated animal, the organism of the untreated animal is able to dissolve large amounts of cholera bacilli, amounts which would otherwise be invariably fatal. This process, as R. Pfeiffer showed, is a specific one, i.e.,

[1] R. Pfeiffer, Zeitschr. Hygiene, Vol. xviii, 1894.

the serum of the guinea pig treated with cholera bacilli transmits an increased solvent power only for cholera bacilli, but not for any other species of bacteria. The active substance of such a bacteriolytic immune serum Pfeiffer called a *specific bactericide*. If we allow some of this specific cholera immune serum to remain for some time outside of the body, e.g. in a bottle, and then test it for solvent properties against cholera bacilli, not in a living body but in a test-tube, we shall find that its power is almost nil. If we add to this serum in the test-tube some fresh peritoneal exudate or some other body fluid, such as serum of a normal, untreated guinea pig, as Metchnikoff first did, we find that this serum has now acquired the power to rapidly dissolve cholera bacilli even in a test-tube. Bordet,[1] in 1895, showed that in order for the specific immune serum to dissolve bacilli in a test tube, it is unnecessary to add fresh normal serum or peritoneal fluid; but that immune serum freshly drawn from the vein is able even under these circumstances to dissolve the bacilli.

Hæmolysis. — Let us now turn for a moment to the development of this subject along other lines. If we go back to the time when blood transfusion was first practised we find it stated that the bloods of different animals transfused into man were more or less directly injurious, and not capable of replac-

[1] Bordet, Annal. Inst. Pasteur, 1895.

ing human blood for this purpose. Landois [1] in a study published in 1875 showed that while transfusion of a foreign blood might prove fatal to an animal the transfusion from a closely related species produced no ill effects. In 1898 Belfanti and Carbone [2] showed that if horses were injected with red blood cells of rabbits, the serum thereafter obtained from the horses would have acquired an appreciable toxicity for rabbits. Shortly after this, Bordet published a very interesting series of experiments. He showed that the serum of guinea pigs after these had been injected several times with 3 to 5 cc. of defibrinated rabbits' blood acquires the property to dissolve rapidly and intensely, in a test-tube, the red blood cells of a rabbit; whereas the serum of a normal guinea pig is incapable of doing this, or does it in only a slight degree. Bordet could further show that this action is a specific one, i.e., the serum of animals treated with rabbit blood acquires this dissolving property only for the red cells of rabbits, not for those of any other species of animal. For the latter, such a serum is no more strongly solvent than the serum of a normal animal. The same property that Bordet had demonstrated in the serum of guinea pigs treated with rabbit blood could now be shown for the sera of all ani-

[1] Landois, Zur Lehre von der Bluttransfusion, Leipzig, 1875.
[2] Belfanti and Carbone, Giorn. della R. Acad. di Med. di Torino, 1898.

mal species treated with blood cells of a different species. We can formulate this as follows: The serum of animals, species *A*, after these have been injected either subcutaneously, intraperitoneally, or intravenously with erythrocytes of species *B*, acquires an increased solvent action for erythrocytes of species *B*, and only for this species.[1] It is therefore a specific action. We call this *hæmolysis*, and the substances which effect the solution of the red cells, *hæmolysins* or *hæmotoxins*.

At about the same time, and independently of Bordet, similar experiments with similar results were published by Landsteiner [2] and v. Dungern.[3] As a result of this work, the acquired toxicity of horse serum, found by Belfanti and Carbone when they treated horses with red cells of rabbits, was explained. The serum of the horses so treated had become *hæmolytic* for rabbit blood, and therefore caused a solution or destruction of the red cells in the living body just as it did in a test-tube.

Nature of Hæmolytic Sera. — In a subsequent study Bordet [4] was able to show that the solvent power of the specific hæmolysins depended on the combined action of two constituents of the specific serum. When the fresh hæmolytic serum was warmed for half an hour to 55° C., it lost its

[1] We shall point out a few exceptions later on.
[2] Landsteiner, Centralblatt Bacteriol. Vol. xxv, 1899.
[3] Von Dungern, Münch. med. Wochenschrift, 1898.
[4] Bordet, Annal. Inst. Pasteur, Vol. xii, 1898.

power. If to this *inactive serum* a very small amount of the serum of a normal guinea pig was added (a serum which of course was not hæmolytic for rabbit red cells), the full hæmolytic power was restored to this inactive serum. In other words, it had been *reactivated* by this addition.

This experiment permits of only one conclusion, namely, that the hæmolytic action of the specific hæmolytic serum depends on two substances. One of these is able to withstand heating to 55° C., and is contained only in the specific serum. The other is destroyed by heating to 55° C., and is contained not only in the specific hæmolytic serum, but also in the serum of normal untreated animals.

Buchner, we have seen, applied the term *alexins* to the constituents of normal serum which were actively destructive to corpuscular elements, bacteria, and other cells with which they came in contact. This term was retained by Bordet to designate that constituent of normal serum which did not withstand heating to 55° C., and which was one of the factors in the hæmolytic process. The other substance, which was found only in the specific serum and which withstood heating to 55° C., he termed *substance sensibilatrice.*

According to Bordet, therefore, the substances required for hæmolysis are the substance sensibilatrice of the specific hæmolytic serum and the alexin which exists even in normal serum. The

action of these two substances Bordet explains by assuming that the red cell is not vulnerable to the alexin; just as, for example, there are certain substances that will not take a dye without the previous action of a mordant. The substance sensibilatrice plays the rôle of mordant. It makes the blood cells vulnerable to the alexin, so that the latter can attack the cells and dissolve them. The alexin he regards as a sort of ferment body with digestive powers.

Bordet says further, that the substance sensibilatrice sensitizes the blood cells not only for the alexin derived from the serum of the same species as that from which it (the substance sensibilatrice) is derived, but sensitizes such cells also for the alexins of normal sera of other species. For example, in the foregoing experiment of Bordet, the substance sensibilatrice derived from the guinea pig by treatment with rabbit blood sensitizes the red blood cells of rabbits not only for the alexin of normal guinea pig blood, but also for the alexins of other normal sera. In another experiment this author showed that rabbit red cells sensitized with an inactive specific hæmolytic serum derived from a guinea pig would dissolve rapidly on the addition of normal rabbit blood. Here, then, the rabbit red cells, sensitized (according to Bordet) by the substance sensibilatrice of the guinea pig, dissolve on the addition of the alexin of their own serum.

The Exciting Agent. — If we now seek to discover the constituent part of the red cell which in the treatment excites in the animal body the production of the specific hæmolysin, we find this to be, according to Bordet and v. Dungern, the stroma of the red cells. This separated from the cell contents and injected into animals will likewise excite the production of specific hæmolytic serum. In opposition to this, Nolf assumes that the stroma excites the production of the above-mentioned agglutinins, and that the production of the substance sensibilatrice is called forth by the contents of the red cells.

Résumé. — Reviewing the important facts we have learned, we find them to be as follows: By means of the treatment of one species of animal with the red cells of a different one, the serum of the first species acquires an uncommonly increased power to dissolve and to agglutinate the red cells of the second species. This increased hæmolytic power shows itself not only in vivo, so that an animal so treated is able to cause red cells injected into it to rapidly dissolve and disappear, but it shows itself also in vitro when the serum of this animal is used. The process consists in the combined action of two substances, that which is excited in response to the injection, the substance sensibilatrice, and the alexin of normal serum.

Analogy between the Bacteriolytic and Hæmolytic Processes. — If we now recall the main points in

cholera immunity the close analogy between this and the subject of hæmolysis is apparent. Just as, when immunizing an organism against cholera bacilli the organism responds with an increased solvent power for those bacteria, so does the organism respond when it is treated, i.e. immunized, with red cells of another species, by increasing the solvent power of its serum for those particular cells. Furthermore, just as the hæmolytic process was seen to depend on the combined action of two substances, one developed in the hæmolytic serum, the other already present in normal serum, so also in the bactericidal process just studied there are two factors. It is easy to understand, therefore, what formerly was not at all clear, why a specific bactericidal serum against cholera, typhoid, or other infectious disease should not act in a test-tube unless there had first been added some normal serum (according to Metchnikoff), or there had been employed a perfectly fresh serum (according to Bordet): simply because in either of these ways the alexin necessary to co-operate with the substance sensibilatrice is introduced. This alexin no longer exists in the immune serum, if this be not perfectly fresh, for we have seen that it decomposes either on warming, or spontaneously on standing. A bactericidal serum, therefore, that has stood for some time is incapable of dissolving bacteria. It is possible, however, to make an old

inactive serum again capable of dissolving bacteria in vitro by adding a little fresh alexin, according to the suggestion of Metchnikoff. In other words, it is thus reactivated. Another obscure point was cleared up by these studies: why a specific bactericidal serum which is inactive in vitro should be intensely active in the living body. This is because in the living body the serum finds the alexin necessary for its working, which is not the case in the test-tube unless fresh normal serum be added. We see from all this that even the first experiments in hæmolysis have served to clear up a number of practical points in an important branch of bacteriology.

Ehrlich and Morgenroth on the Nature of Hæmolysis. — In continuing the study of hæmolysins we must note particularly the researches of Ehrlich and Morgenroth.[1] These authors asked themselves the following questions: (1) What relation does the hæmolytic serum or its two active components bear to the cell to be dissolved? (2) On what does the specificity of this hæmolytic process depend? Ehrlich was led to these researches particularly by his so-called Side-chain Theory, which we shall examine in a moment.

He made his experiments with a hæmolytic serum that had been derived from a goat treated

[1] Ehrlich and Morgenroth. See the various papers in "Collected Studies on Immunity," Wiley and Sons, New York, 1906.

with the red cells of a sheep. This serum, therefore, was hæmolytic specifically for sheep blood cells; i.e., it had increased solvent properties exclusively for sheep blood cells.

Basing his reasoning on his side-chain theory, Ehrlich argued as follows: "If the hæmolysin is able to exert a specific solvent action on sheep blood cells, then either of its two factors, the substance sensibilatrice of Bordet or the alexin of normal serum, must possess a specific affinity for these red cells. It must be possible to show this experimentally." Such in fact is the case, and the experiments devised by him are as follows:

Experiment 1. — Ehrlich and Morgenroth, as already said, experimented with a serum that was specifically hæmolytic for sheep blood cells. They made this inactive by heating to 55° C., so that then it contained only the substance sensibilatrice. Next they added a sufficient quantity of sheep red cells, and after a time centrifuged the mixture. They were now able to show that the red cells had combined with all the substance sensibilatrice, and that the supernatant clear liquid was free from the same. In order to prove that such was the case they proceeded thus: To some of the clear centrifuged fluid they added more sheep red cells; and, in order to reactivate the serum, a sufficient amount of alexin in the form of normal serum was also added. The red cells, however, did not dissolve —

there was no substance sensibilatrice. The next point to prove was that this substance had actually combined with the red cells. The red cells which had been separated by the centrifuge were mixed with a little normal salt solution after freeing them as much as possible from fluid. Then a little alexin in the form of normal serum was added. After remaining thus for two hours at 37° C. these cells had all dissolved.

In this experiment, therefore, the red cells had combined with all the substance sensibilatrice, entirely freeing the serum of the same. That the action was a chemical one and not a mere absorption was shown by the fact that red blood cells of other animals, rabbits or goats for example, exerted no combining power at all when used instead of the sheep cells in the above experiment. The union of these cells, moreover, is such a firm one that repeated washing of the cells with normal salt solution does not break it up.

The second important question solved by these authors was this: What relation does the alexin bear to the red cells? They studied this by means of a series of experiments similar to the preceding.

Experiment 2. — Sheep blood was mixed with normal, i.e. *not* hæmolytic, goat serum. After a time the mixture was centrifuged and the two portions tested with substance sensibilatrice to determine the presence of alexin. It was found that in

this case the red cells acted quite differently. In direct contrast to their behavior toward the substance sensibilatrice in the first experiment, they now did not combine with even the smallest portion of alexin, and remained absolutely unchanged.

Experiment 3. — The third series of experiment was undertaken to show what relations existed between the blood cells on the one hand, and the substance sensibilatrice and the alexin on the other, when both were present at the same time, and not, as in the other experiments, when they were present separately. This investigation was complicated by the fact that the specific immune serum very rapidly dissolves the red cells for which it is specific, and that any prolonged contact between the cells and the serum, in order to effect binding of the substance sensibilatrice, is out of the question. Ehrlich and Morgenroth found that at 0° C. no solution of the red cells by the hæmolytic serum takes place. They therefore mixed some of their specific hæmolytic serum with sheep blood cells, and kept this mixture at 0°–3° C. for several hours. No solution took place. They now centrifuged and tested both the sedimented red cells and the clear supernatant serum. It was found that at the temperature 0°–3° C. the red cells had combined with all of the substance sensibilatrice, but had left the alexin practically untouched.

It still remained to show the relation of these two substances to the red cells at higher temperatures. At 37°–40° C., as already mentioned, hæmolysis occurs rapidly, beginning usually within fifteen minutes. It was possible, therefore, to leave the cells and serum in contact for not over ten minutes. Then the mixture was centrifuged as before. The sedimented blood cells mixed with normal salt solution showed hæmolysis of a moderate degree. The solution became complete when a little normal serum was added. The supernatant clear fluid separated by the centrifuge did not dissolve sheep red cells. On the addition, however, of substance sensibilatrice it dissolved them completely.

So far as concerns the technique of the experiments, I should like to observe that the addition of red cells in this as well as in all the following experiments was always in the form of a 5% mixture or suspension in 0.85%, i.e. isotonic, salt solution.

The significance of the last of the above-cited experiments is at once apparent. It is that the substance sensibilatrice possesses one combining group with an intense affinity (active even at 0° C.), for the red cell, and a second group possessing a weaker affinity (one requiring a higher temperature) for the alexin.

Nomenclature. — In place of the name substance

sensibilatrice Ehrlich first introduced the term *immune body*, later on he called it the *amboceptor*. In the following pages we shall use the term *immune body*, as this had already been used by R. Pfeiffer to designate the same substance in bactericidal serum. Other names proposed for this substance have been *substance fixatrice* by Metchnikoff, *copula*, *desmon*, *preparator* by Müller. Instead of the name alexin, Ehrlich now uses the term *complement* in order to express the idea that this body completes the action of the immune body.

In contrast to the specific affinity which the red cells possess for the immune body, these cells possess no affinity whatever for the alexin, as has been shown by the second of Ehrlich's experiments. The alexin, therefore, possesses no combining group which can attach itself directly to the red blood cell. It acts on these cells only through an intermediary, the immune body, which therefore must possess two binding groups one which attaches to the red blood cell and the other to the alexin of normal serum. As already stated, the group which attaches to the red blood cell possesses a much stronger affinity than that which combines with the alexin. This follows from the last two experiments of Ehrlich before cited, in which he showed that at the lower temperature, and with both substances present with the blood cells, only the immune body combined with the cells, while

the alexin remained uncombined. At the higher temperature the alexin also exerted its affinity, for then the red cells combined with all the immune body and with part of the alexin. We saw that after a time the red cells partially dissolved, but that complete solution occurred only after some fresh alexin had been added. This showed that although the red cells had combined with all the immune body necessary for their solution, they had been unable to bind all the alexin necessary. We may say, therefore, that that group of the immune body which combines with the red cell has a stronger affinity than that which combines with the alexin.

Rôle of the Immune Body. — According to Ehrlich, then, the rôle of the immune body consists in this, that it attaches itself to the red cell on the one hand, and to the complement on the other, and in this way brings the digestive powers of the latter to bear upon the cell, the complement possessing no affinity for the red cell. Immune body and complement have no very great affinity for each other. At 0° C. they may exist in serum side by side, and they combine only at higher temperatures.

The amount of immune body which combines with the red cells may vary greatly, as the experiments of Bordet and of Ehrlich clearly show. Some red cells combine with only just enough immune body to effect their solution. Others are

able to so saturate themselves with immune body that they may have a hundred times the amount necessary for their solution.

On what the Specificity Depends. — From the preceding it follows that the specific action of the hæmolytic sera, and, I may at once add, of the bactericidal sera also, is due exclusively to the immune body. This possesses a combining group which is specific for the cells with which the animal was treated; e.g., the combining group of an immune body produced by treatment with rabbit blood will fit only to a certain group in the blood cells of rabbits; an immune body produced by treatment with chicken blood will fit only to parts of the red cells of chickens; one produced by treating an animal with cholera bacilli will fit only to this species of bacteria and combine only with the members of it. Keeping to the well-known simile of Emil Fischer, the relation is like that between lock and key, each lock being fitted only by a particular key.

To repeat — for the point is of the greatest importance — the rôle of the immune body consists in tying the complements of normal serum, which have no affinity for the red cells or for the bacteria, indirectly to these cells so that their solution and digestion may be effected by the complements. In other words, the immune body serves to concentrate on the corpuscular element to be dis-

solved all the widely distributed complement found in normal serum.

The relation existing between complement, immune body (i.e., amboceptor) and erythrocyte is shown in the accompanying figure reproduced after Levaditi, a pupil of Ehrlich.

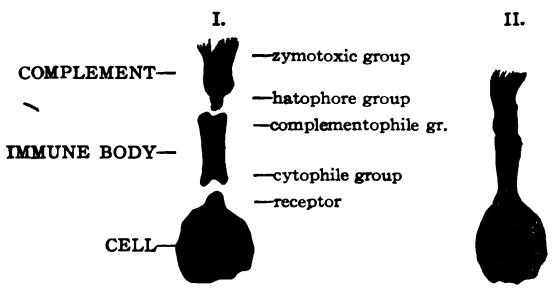


FIG. 7

Difference between a Specific Serum and a Normal One. — The difference, then, between a specific hæmolytic or a specific bactericidal serum and a normal one consists in this — *that the specific serum contains an immune body which is specific for a certain cellular element and by means of which the complement present in all normal serum can be concentrated on this element to cause its solution.* We shall return to this subject later.

Diverging Views of Ehrlich and Bordet. — Now if we recall the first experiments of Bordet and his conclusions respecting the manner in which the factors concerned acted, we shall at once see how

Ehrlich and Bordet differ. Bordet assumes that the substance sensibilatrice (the immune body) acts as a kind of mordant on the red cells or bacteria, sensitizing these to the action of the alexin (complement). According to Ehrlich, however, the process is not analogous to a staining process, but follows definite laws of chemical combination, there being, in fact, no affinity whatever between the complement and the blood cells or bacteria. Furthermore, according to this authority, the complement always acts through the mediation of the immune body, which possesses two combining groups; one, the cytophile group, combining with the cell, and another, the complementophile group, combining with the complement. Both observers have devised a series of ingenious experiments to support their views. But as these can interest only the specialist, we shall omit their discussion here. For such details the original articles may be consulted.

The Side-Chain Theory Applied to these Bodies. — All of the specific relations which, in a previous chapter, we saw existed between toxin and antitoxin, Ehrlich and Morgenroth in their experiments above noted found existed also between immune body and the specific blood cell. The immune body must therefore possess a haptophore group which fits exactly to certain receptors or side chains of the red cells, just as the anti-body

according to the side-chain theory possesses a group that fits exactly into the specific combining group — i.e., haptophore group — of the toxin or toxoid used for exciting the immunity.

If, for example, we produce a hæmolytic serum specific for red cells of a rabbit by injecting an animal with these cells, the haptophore groups of this serum, i.e., the free side chains thrust off, must possess specific combining relations with the red cells of rabbits. That such is the case in the hæmolytic immune serum we saw from the experiments of Ehrlich and Morgenroth.

In consequence of all this, Ehrlich widened the application of his side-chain theory so as to include not only the production of antitoxin but also the production of bactericidal, hæmolytic, and other immune bodies. He expressed this somewhat as follows: *If any substance, be it toxin, ferment, constituent of a bacterial or animal cell, or of animal fluid, possess the power by means of a fitting haptophore group to combine with side chains (receptors) of the living organism, the possibility for the overproduction and throwing off of these receptors is given, i.e., the possibility to produce a corresponding anti-body.*

Specific anti-bodies in the serum as a result of immunizing processes can only be produced, therefore, by substances which possess a haptophore group and which, in consequence, are able to form a

firm union with a definite part of the living organism, the receptor. This is not the case with alkaloids, e.g., morphine, strychnine, etc., which according to Ehrlich enter into a loose union, a kind of solid solution with the cells. It is for this reason that we are unable to produce any anti-bodies in the blood serum against these poisons. Ehrlich says further that all of the substances taking part in the production of immunity, including of course complement and immune body, have certain definite affinities for each other, and in order to act they must fit stereochemically to each other.

As we have already seen, we are able by means of the injection of a variety of substances or cells to produce a similar variety of immune bodies in the serum. Thus we can immunize a rabbit so that its serum will possess specific hæmolytic bodies against the red cells of guinea pigs, goats, chickens, and oxen and specific bactericidal bodies against cholera and typhoid bacilli, etc., and as we shall see, still other groups of anti-bodies.

Multiplicity of Complements. — Under these circumstances an important question presents itself: Is there in normal serum one single complement which completes the action of all these various immune bodies, one, for example, which in the above illustration will fit all the hæmolytic immune bodies as well as all the bactericidal ones, or are there a great many different complements?

Ehrlich, as a result of his experimental work with Morgenroth, claims that the latter is the case; namely, that it takes a different complement to fit the immune body specifically hæmolytic for guinea pig blood than it does to fit that specific for chicken blood.

Bordet, on the other hand, assuming that the immune body plays the rôle of mordant, believes as does also Buchner, that there is but one single complement in the serum. According to him, this complement is able to dissolve blood cells as well as bacteria after these have been sensitized by their specific immune body. Each of these authors supports his claims by means of ingenious experiments, for the details of which, however, we must refer to the original articles, as they require the knowledge of a specialist for their comprehension. We shall, however, give one of Bordet's [1] experiments on this point in some detail since it has found extensive application in another direction.

The Bordet-Gengou Phenomenon. — Bordet sensitized blood corpuscles with appropriate amboceptors, and then exposed them to the action of a freshly drawn normal serum. If now he waited for the occurrence of hæmolysis and then added *sensitized* cells (bacteria or blood corpuscles of a different species), the latter remained entirely unchanged, although the serum that had been used as complement was capable in its original con-

[1] Bordet and Gengou, Annal. Inst. Pasteur. Vol. xv, 1901.

dition of destroying these also. When fresh serum was first brought into contact with sensitized *bacteria*, similar results were obtained. The blood corpuscles subsequently added did not then undergo hæmolysis. *If such an action on one of the sensitive substrata has once taken place, the active sera, as a rule, are deprived of all their complement functions*, from which Bordet concludes that the destruction of the most varied elements by one and the same serum must be due to a single complement.

It may be said in passing that Ehrlich admits the correctness of the above experimental results, but brings forward additional arguments showing that Bordet's interpretation as to the existence of only a single complement cannot be accepted.

This experiment of Bordet is usually spoken of as the "Bordet-Gengou phenomenon" and is now used largely in determining whether or not a given serum possesses certain amboceptors. The serum to be tested is first heated and then mixed with a small quantity of fresh normal serum (complement) and with an emulsion of the bacterium whose amboceptors it is desired to discover. After standing for six hours at room temperature, red blood cells previously treated with heated hæmolytic serum are added. If there is no hæmolysis it is held to mean that the complement in the fresh serum which was suitable for lysis of properly prepared blood corpuscles, has been absorbed by the bacteria by reason of the presence of specific amboceptors in the serum tested.

Wassermann[1] has recently successfully applied this method in measuring the amboceptor content of specific

[1] Wassermann, Neisser and Bruck, Deutsche med. Wochenschr, 1906; Wassermann and Plaut, Ibid.

meningococcus sera and also in diagnosing syphilitic antigens and antibodies.

Neisser and Sachs[1] have recently described a procedure for the forensic diagnosis of blood stains. The principle of this is the same as in the preceding although in so far as a specific *precipitin* serum is made use of, the procedure is really modelled after the "Gengou-Moreschi" phenomenon.

If human blood serum is mixed with a specific human precipitin serum derived from rabbits, it will be found that the mixture binds complement. Hæmolysin subsequently added is unable to dissolve its specific red blood cells, owing to this locking up of the complement. Only the serum of monkeys has a similar effect. The amount required is extremely minute, $\frac{1}{100000}$ to $\frac{1}{1000000}$ cc. human blood or monkey blood sufficing. Extracts of human blood stains will also produce the desired effect. The authors believe that the immunization with human blood serum gives rise not only to precipitins but also to amboceptors which then are able to unite with their corresponding unformed albuminous bodies and so bind complement. Others are of the opinion that the complement is bound by the precipitin-precipitum combination.

The test is extremely delicate and has been found trustworthy by a number of investigators. In view of the importance of such tests in medico-legal cases, Neisser and Sachs suggest that it should always be used in addition to the well known Wassermann-Uhlenhuth precipitin test.

Normal Serum, its Hæmolytic and Bacteriolytic Action. — Inquiring now into the essential differ-

[1] Neisser and Sachs, Berliner klin Wochenschrift, 1905.

ence between a specific hæmolytic or bactericidal serum and a normal one, we must first of all study the behavior of normal serum toward foreign red cells and bacteria. It has long been known to physiologists that fresh normal serum of many animals has the power to dissolve blood cells of another species. This was studied especially by Landois. One-half to one c.c. of normal goat serum, for example, is able to dissolve 5 c.c. of a 5% mixture (in normal salt solution) of rabbit or guinea pig red cells. In the same way, these red cells are dissolved by the sera of oxen, of dogs, etc. This *normal globulicidal* property of the serum corresponds to another which fresh normal serum was found to possess, namely, the property to dissolve appreciable quantities of many species of bacteria. This analogy was pointed out by Fodor, Nutall, Nissen, and especially by Buchner. We call this the *bactericidal* property of fresh normal serum.

This property is well illustrated by the following protocol from Park.

No. of bacteria in 1 cc. fluid.	Amount of serum added.	Approximate number alive after being kept at 37° C.		
		One hour.	Two hours.	Twenty-seven hrs.
30,000	0.1 cc.	400	2	0
100,000	0.1 cc.	5,000	1,000	200,000
1,000,000	0.1 cc.	400,000	2,000,000	10,000,000

It is at once apparent that the number of bacteria introduced is an important factor, the normal serum being able to kill off only a certain number.

Buchner, as we have already seen, had studied this bactericidal action carefully and ascribed the action to a substance found in all normal serum, which he called *alexin*. According to his experiments, this is a very unstable substance, decomposing spontaneously on standing or on heating for a few minutes to 55° C., or readily on the action of chemicals. According to this author all the globulicidal and bactericidal functions of normal serum are performed by this one substance, the alexin.

Active and Inactive Normal Serum. — Ehrlich and Morgenroth now took up the study of the hæmolytic action of normal serum. They sought particularly to discover whether in normal serum the hæmolytic property depended on the action of a single substance, the complement (Buchner's alexin), or whether here as in the specific hæmolytic serum it depended on the combined action of two substances. For this purpose they used guinea-pig blood, which is dissolved by normal dog serum. If this serum was heated to 55° C., it lost its hæmolytic power. It was necessary now to show that in this inactive dog serum there remained a second substance which could be reactivated after the manner of reactivating an old specific hæmolytic serum. This had its difficulties, for they could not add normal dog serum. This, as we saw, is already hæmolytic for guinea-pig

blood. " Possibly," said they, " there exists a complement of another animal which will fit the hypothetical second substance of this dog serum." This proved to be the case, the complement of guinea-pig blood fulfilling the requirements. If they added to the inactive normal dog serum about 2 c.c. normal guinea-pig serum the hæmolytic property was restored and the guinea-pig red cells dissolved completely. This can only be explained by assuming that in guinea-pig blood there exists a complement which happens to fit the haptophore group of the second substance or inter-body, of the normal dog serum. This combination of guinea-pig blood, inactive normal dog serum, and a reactivating normal guinea-pig serum is well adapted to demonstrate the existence in normal dog serum of an inter-body; for the guinea-pig serum should be the best possible preservative for the guinea-pig red cells. The hæmolysis following the addition of this serum shows positively the existence of a substance in the dog serum which has acted with something in the guinea-pig serum.[1]

[1] Of such combinations, i.e., combinations in which a complement derived from the same animal from which the red cells are derived fits to the inter-body of other species of animals, causing the solution of red cells of the latter, Ehrlich and Morgenroth found still other examples. For instance, guinea-pig blood, inactive calf serum, guinea-pig serum; goat blood, inactive rabbit blood, goat serum; sheep blood, inactive rabbit blood, sheep serum; guinea-pig blood, inactive sheep serum, guinea-pig serum.

Inter-body and Complement. — We see, then, that the hæmolytic action of normal sera depends, just as that of the specific hæmolytic sera, on the combined action of two bodies: one, the *inter-body*, which corresponds to the immune body of the specific sera, and a second or *complement.* In speaking of the constituents of *normal* serum, Ehrlich and Morgenroth prefer to use this term *inter-body* to distinguish it from the *immune bodies* of *specific* hæmolytic sera.

Action not Entirely Specific. — It has also been found that there frequently exist normal sera which are hæmolytic not only for one species of red cell, but for several. We saw, for instance, that normal goat serum dissolved the red cells of guinea pigs and rabbits. The question now arises, Is this property of normal goat serum due to two inter-bodies existing in the serum side by side, one fitting the red cells of the guinea pig, the other those of the rabbit? Ehrlich and Morgenroth answered this in the affirmative, for in the following experiment they succeeded in having each of the two inter-bodies combine with its respective cell. To some inactive normal goat serum they added rabbit blood and centrifuged the mixture. To the separated clear fluid they again added some rabbit red cells as well as normal horse serum to reactivate the mixture. Horse serum is not hæmolytic for rabbit red cells. The mixture remained

unchanged, no hæmolysis taking place. If, however, they added some of this normal horse serum to the centrifuged red cells, the latter immediately dissolved. Now, to the clear centrifuged fluid, which as we have seen would not dissolve rabbit red cells, they added guinea-pig red cells and again some normal horse serum to reactivate the mixture. The guinea-pig red cells all dissolved. This proved conclusively that in the normal goat serum there had existed two specific inter-bodies. One, for rabbit red cells, had been tied by these cells and carried down with them in centrifuging; the other, specific for guinea-pig red cells, had remained behind.

Multiplicity of the Active Substances. — These investigators were able to prove still more in regard to the multiplicity of the substances in normal serum which are concerned in hæmolysis. They showed that beside the two inter-bodies just mentioned there existed in goat serum two specific complements, one for each inter-body, and they were able by means of Pukall filters to separate these two. In this filtration the complement fitting the inter-body for rabbit blood remained behind for the greater part, while that fitting the inter-body for guinea-pig blood mostly passed through.

Whereas then, according to Buchner, only one substance, the alexin, is concerned in the hæmolytic action of this normal goat serum these experi-

ments of Ehrlich and Morgenroth show us four substances, viz., two inter-bodies and two complements. This at once makes clear the opposing views of these authorities. But the number of active substances in normal serum is still greater, for in the experiments of the last-named authors it often happens that a specific inter-body shows itself to be made up of several inter-bodies, all, to be sure, fitting the same specific red cell, but differing from each other by their behavior toward different complements. Ehrlich, therefore, regards the substances concerned in hæmolysis which occur in normal serum to be of great number and variety. Buchner and Bordet, on the other hand, assume that only one substance is concerned.

Difference between a Normal and a Specific Immune Serum. — Practical Application. — Returning now to the question of the difference between a specific immune serum and a normal one, we find this to be as follows: Normal serum contains a great variety of inter-bodies, in very small amounts, and a considerable amount of complements. In immune serum, on the other hand, the amount of a specific inter-body, the one which fits the haptophore group of a certain cell, is enormously increased. This specifically increased inter-body, it will be remembered, is called the immune body. The complement, as shown by v. Dungern, Bordet, Ehrlich, and Morgenroth and Wassermann, is in no way

increased by the immunizing process. The increase affects solely the immune body. It is therefore possible to have a serum which contains more immune body than complement to satisfy it, and if we withdraw such a serum from an animal we shall find that it contains some free immune body. This serum can only then exert its full power when the full amount of complement is present, i.e., when some normal serum is added. If we treat a rabbit with the red cells of an ox, as v. Dungern did, we shall obtain a serum which is hæmolytic for ox blood. Of this freshly drawn serum 0.05 c.c. suffice to dissolve 5.0 c.c. of a 5% mixture of ox blood. If now we add to this hæmolytic serum a little normal rabbit serum, we shall find that only one-tenth of the amount of serum is required; i.e., only 0.005 c.c. to dissolve the same quantity of ox blood. This means that through the addition of the rabbit serum, which, of course, is not hæmolytic for ox blood, a sufficient amount of complement was added to enable all the immune body of the specific serum to act. This specifically increased power of the immune serum to act on certain definite cells depends on the fact that the immune body resulting from the immunizing process concentrates the action of the complement scattered through the serum, on cells for which it has definite affinities. If 2 c.c. of normal guinea-pig serum are able to dissolve, we will say,

5 c.c. of a 5% defibrinated rabbit-blood mixture, and if we find that after the immunizing process 0.05 c.c. of the guinea-pig serum suffice to dissolve the same amount of rabbit blood, we conclude that through this process the inter-body, i.e. the immune body, has been increased forty times. We know that the complement has not been increased, but this is now able to act by means of forty times increased combining facilities. This increase, however, is exclusively for rabbit-blood cells. In a bactericidal immune serum this specific increase is sometimes as much as 100,000 times that of normal serum.

The practical idea to be gained from this for the therapy of infectious diseases is this: that with the injection of an immune serum we supply only one of the necessary constituents to kill and dissolve the bacteria, and that is the immune body.

We do not, however, supply the second, i.e. the complement, for this we have seen is not increased by the immunizing process. As matters stand, then, the use of a specific immune serum for therapeutic purposes assumes that the complement which fits exactly to the immune body and which is essential for the latter's action will be found in the organism to be treated. Since in certain infectious diseases the required complement is present in too small amounts in the organism, Wassermann

suggested that the curative power of many bactericidal sera might be increased by the simultaneous injection of the sera of certain normal animals in order thus to gain an increased amount of complement; but we shall soon see that this procedure, while of great value in animal experiments, presents certain difficulties.

Nature of the Immune Body — Partial Immune Bodies of Ehrlich. — Turning now to a closer study of the nature of the immune body, we again find a difference of opinion. Whereas Bordet, Metchnikoff, and Besredka assume each immune body to be a single definite substance, Ehrlich and Morgenroth as a result of their experiments hold to a plurality of bodies.

These authors say that each immune body is built up of a number of *partial-immune bodies*, a point to which we have already alluded. In support of this view they offer the following experiment. On immunizing a rabbit with ox blood, they obtained a serum hæmolytic not only for ox blood but also for goat blood; on immunizing a rabbit with goat blood they obtained a serum hæmolytic for goat blood and ox blood.[1]

The conditions present can be readily understood by reference to Fig. 7, which represents schematically three portions of the combining groups

[1] We have already called attention to these exceptions to the rule of specific action.

of the blood cells. Of these α is present only in the ox-blood cells, ψ only in the goat-blood cells, and β in both. If a rabbit is injected with ox blood, the immune bodies corresponding to groups α and β will be formed. On subjecting such a serum to absorption with ox-blood cells we shall find that these, by means of their α and β groups will be able to absorb *all* the immune bodies, whereas goat-blood cells will in a similar test absorb only the immune

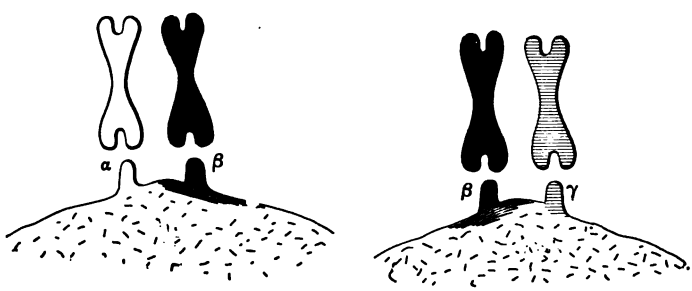


FIG. 7

body of portion β, leaving the immune body of portion α in solution.

According to Ehrlich's theory, then, the red cells of the ox possess certain receptors which are identical with receptors possessed by the goat red cells. From this it follows that in a single red cell there are several or many groups each of which is able, when it finds a fitting receptor, to take hold of a

single immune body. Ehrlich and Morgenroth, therefore, claim that the immune body of a hæmolytic serum is composed of the sum of the partial immune bodies which correspond to the individual receptors used to excite the immunity. It may be assumed, then, that not all of the combining groups of a cell, be this a blood cell or a bacterium, will find fitting receptors in every animal organism, and that therefore not all the possible partial immune bodies will be equally developed. In one animal there may be receptors which are not present in another, and in this way there might be a different variety of partial immune bodies in the two animals. This would lead to the possibility of the occurrence of immune bodies, for the same species of blood cell or bacterium, differing from each other in the partial immune bodies composing them, according to the variety of animals used in preparing the serum.

Metchnikoff's Views — Practical Importance of the Point. — This view is directly opposed to that of Metchnikoff and Besredka, who believe that a certain immune body, e.g. one specific for ox blood, is always the same no matter from what animal it is derived. The point is not merely theoretical, but under certain circumstances of great practical importance. If we believe, as Ehrlich does, that the immune body differs according to the species of animal from which it is derived, i.e., that it is made

up of different partial-immune bodies, then we must admit that we have better chances for finding fitting complements if we make use of immune bodies derived from a variety of animals. We would, for instance, be likely to achieve better results in treating a typhoid patient with a mixture of specific bactericidal typhoid sera derived from a variety of animals than if we used a serum derived only from a horse. For in such a mixture of immune bodies the variety of partial-immune bodies must be very great and the chances that the complements of the human body will find fitting immune bodies, and so lead to the destruction of the typhoid bacilli, are greatly increased. Ehrlich and his pupils have actually proposed such a procedure in the use of bactericidal sera for therapeutic purposes.[1]

Support for Ehrlich's View. — Besides the above experiments we possess others which support the theory that the immune body is not a simple but a compound substance. v. Dungern had already shown that following the treatment of an animal with ciliated epithelium from the trachea of an ox, there were developed immune bodies which acted not only on the ciliated epithelium but also on the red cells of oxen. We must assume, therefore, that

[1] Reasoning along similar lines, namely, that the human complement must fit the immune body of the therapeutic serum, Ehrlich has also proposed that these bactericidal sera be derived from animals very closely related to man, e.g., apes, etc.

the ciliated epithelium and the red cells of the ox possess common receptors. Analogous to this is the action of the immune body resulting from the injection of spermatozoa, as was pointed out by Metchnikoff and Moxter.

We see, then, that the specific action of immune bodies is not so limited as to apply only to the cells used in the immunizing process, but extends to other cells which have receptors in common with these.[1]

Coming now to the question as to what part of the cell it is which excites the production of the hæmolytic immune body, we find this, according to v. Dungern, to be the stroma of the red cells. If this be so, it must be the stroma which combines with the immune body. Nolf, however, claims that the cell contents are factors in the production of the immune body. So far as concerns the site in the organism where the substances used in immunizing find their receptors, this is not known for the hæmolytic immune body.

For the bactericidal immune bodies of cholera and typhoid the researches of Pfeiffer, Marx, and others show that the chief site of production is in

[1] The same holds good for the agglutinins and the precipitins still to be studied. In these the action extends also to closely related cells and bacteria, or in the case of the precipitins to closely related albumins, as these possess a number of receptors which are common to them and to the cells or substances used for immunizing.

the bone-marrow, spleen, and lymph bodies. Wassermann's experiments on local immunity indicate that the site of infection determines largely the site of the development of the immune bodies.

Antihæmolysins: their Nature — Anti-complement or Anti-immune Body? — A further step in the study of hæmolysins is one discovered independently by Ehrlich and Morgenroth on the one hand, and Bordet on the other. These authors succeeded in producing an *antihæmolysin*. The procedure is closely related to the results gained by immunization against bacterial poisons. A specific hæmolysin, one, for example, specific for rabbit blood, derived by treating a guinea pig with rabbit red cells, is highly toxic to rabbits. Injected into the animals intravenously in doses of 5 c.c. it kills the animals acutely, causing intra vitam a solution of the red cells. Such a hæmolytic serum, then, acts the same as a bacterial poison, and it is possible to immunize against this just as well as against a bacterial poison. For example, to keep to our illustration, rabbits are injected first with very small doses of this specific hæmolytic serum. The dose is gradually increased until it is found that the animal tolerates amounts that would be absolutely fatal to animals not so treated. If some of the serum of this animal is now abstracted and added to the specific hæmolytic serum, it is found that the power of the latter will be inhibited. This shows that an

antihæmolysin has been formed. As we know that the action of the hæmolysin depends on the combined action of two substances, the immune body and the complement, the question arises to which of these two the antihæmolysin is related. Is it an anti-immune body or an anti-complement? A study of this question shows that both these substances are apparently present. In the serum of the rabbit treated with specific hæmolysin, both an anti-immune body and an anti-complement have been found. For the details of the experiments of Ehrlich and Morgenroth and of Besredka, which demonstrated this, I must refer to the original articles. The first-named authors were further able to show that the action of the anti-complement depended on a haptophore group which it possessed, enabling it to combine with the haptophore group of the complement, thus satisfying this and hindering its combination with the complementophile group of the immune body.

Anti-complement. — Since the complements are constituents of normal serum, it should be possible to produce anti-complements by injecting animals merely with normal serum; and they can, in fact, be so produced. If rabbits are treated by injecting them several times with normal guinea pig serum, a serum may be obtained from these rabbits which contains anti-complements against the complements of normal guinea-pig serum. A serum

obtained in this way of course contains only one of the antihæmolytic bodies, the anticomplement, and not the antiimmune body. This is because normal serum is too poor in immune body (inter-body) to excite the production of any antiimmune body.

If to a hæmolytic serum derived from guinea pigs we add an anticomplement serum derived, as just stated, from rabbits, and containing an anticom-

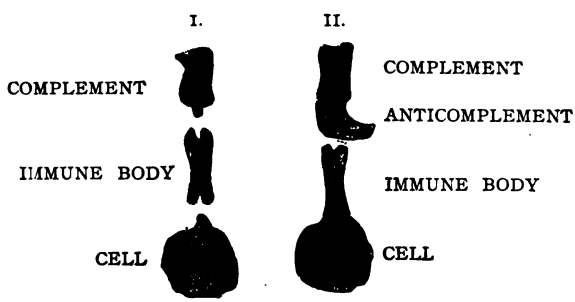


FIG. 8. (After Levaditi.)

plement specific for guinea-pig complement, the hæmolytic action of the former will be inhibited, for the reason that the complement necessary for the hæmolysis to take place has been bound by the anticomplement. (See Fig. 8.) One must, however, observe the precaution to heat the anticomplement serum of the rabbit to 55° C. before so mixing it, in order to destroy the complement which it contains and which would otherwise reactivate the guinea-pig immune body.

From the foregoing we see that either anti-immune body alone, or anticomplement alone, is able to inhibit the hæmolytic action. Hæmolysis cannot take place when either of the two necessary factors is bound and prevented from acting.[1]

The anticomplements are specific bodies, i.e., an anticomplement combines only with its specific complement. Thus an anticomplement serum derived from rabbits by treatment with guinea-pig serum combines only with the complement of normal guinea-pig serum, not, however, with the complements of other animals. Exceptions to this are those cases in which the complement of the other species possess receptors identical with those of the first.

In order that a normal serum of species *A*, injected into species *B*, produce anticomplements there, the side-chain theory demands that the complements of *A* find fitting receptors in species *B*. According to Ehrlich, however, normal serum contains many different complements and not merely a single one. Under the circumstances, it is easily possible that only a few of the complements in the

[1] By treating animals with normal sera of certain other species, it is possible to produce not only anti-complements, but also specific anti-bodies against certain other constituents of normal serum. These are, for example, anti-agglutinins, which inhibit the action of the hæmagglutinins of normal serum, and anti-precipitins, which we shall discuss later.

serum of *A* find fitting receptors in species *B*. We shall then obtain an anticomplement serum which inhibits the action of some, but not of all the complements of species *A*. Thus it might inhibit the action of a complement fitting to a certain bactericidal immune body and not of one contained in the same serum which fitted a certain hæmolytic immune body, etc.

Auto-anticomplements. — A question of great practical importance now arises. Is it possible under certain conditions for an organism to manufacture within itself anticomplements against its own complements, i.e., *auto-anticomplements?* The complements, owing to their ferment-like digestive power, must play an important rôle in the living organism; for this concerns itself not only with the destruction of bacteria, etc., an important factor in the natural immunity against diseases, but also, according to Ehrlich, Buchner, and Wassermann, with the solution and digestion of all kinds of foreign albuminous bodies which enter the organism. Any inhibition of this important function would therefore be followed by severe disturbances, particularly, however, by a decreased resistance against infectious diseases. Wassermann succeeded in demonstrating that animals injected with anti-complements to tie up their complements were much less resistant to certain infectious diseases.

The *spontaneous* development in an animal of

auto-anticomplement, i.e., substances developed within the organism against its own complements, has not yet been demonstrated. Ehrlich and Morgenroth were able to excite the production of an auto-anticomplement in a rabbit by treating the animal in a certain way. Ordinarily, normal rabbit serum is slightly solvent for guinea-pig blood. If the rabbits are treated with goat serum, the rabbit serum loses this solvent power for guinea-pig red cells. Even if fresh, normal rabbit serum is now added, hæmolysis does not take place, although we know that this fresh serum is hæmolytic. This shows that in the serum of the rabbit treated with goat blood, an anticomplement has been formed which combines with the complement of normal rabbit blood, for it was able to inhibit the action of the complement of the normal, freshly added rabbit serum. In the rabbit's body, then, as a result of this procedure, an anticomplement has been formed against the complement of its own serum, a true auto-anticomplement.

Now, according to the side-chain theory, there are no receptors in an organism for the complements of the same organism. The formation of these auto-anticomplements, according to Ehrlich, can only be explained by assuming that in normal goat serum there are present complements which are almost identical with those of the rabbit serum, but which differ from them in that they find recep-

tors in the rabbit serum whose haptophore group fits to their own.

Fluctuations in the Amount of the Active Substances in Serum. — As already said, we have thus far been unable to show that the complements of an organism are decreased through the action of spontaneously formed anticomplements. We have, however, come to know certain conditions under which there may be a decrease of certain complements in normal serum. Ehrlich and Morgenroth showed that in rabbits poisoned with phosphorus and in whom, therefore, the liver was badly damaged, the serum on the second day (the height of the disease) had lost its power to dissolve guinea-pig blood, and that this was due to a disappeareance of the complement. Metchnikoff also reported that in an animal suffering from a continuing suppurating, process, the complement had fallen considerably in amount. Especially interesting are the experiments of v. Dungern, who showed that animal cells, hence emulsions of fresh organs, are able to attract and combine with complements.

Even more important than the question of a decrease in complements, or an inhibition of their action, is that of the possibility to artificially increase them. A number of authors, among them Nolf and Müller, have answered this question in the affirmative. They believe they have noticed such an increase following the injection of an animal with

all sorts of substances, such as normal serum of another animal, sterile bouillon, etc. v. Dungern, Wassermann and others, have not been able to convince themselves of the possibility of such a definite increase. Wassermann tried to excite the increased production of complement by injecting guinea pigs for some time with anticomplement. This being the opposite of the complement, he hoped to be able by immunizing to excite an increase of the complements. In this he was unsuccessful, though of course it may be possible with another species of animal.

Despite all this, we must believe that the amount of complement, as well as the amount of other active substances of the blood, inter-bodies, agglutinins, antitoxins, ferments, antiferments, etc., is subject to great fluctuations even in the same individual, a constant change going on within the organism. Ehrlich, in particular, has pointed out these individual and periodic variations and has insisted on their importance. Very likely, under circumstances of which we now know very little, these substances are at certain times produced in greater amounts, at other times in lesser; sometimes they may be absent entirely in an individual in whom they were previously present. For example, the serum of a dog will at times dissolve the red cells of cats, rabbits, and guinea pigs, at other times not. Furthermore, the serum of one and the same animal may

possess specific hæmolytic properties for certain cells, and later on may lose this property entirely. In human serum these same individual and periodic variations may be demonstrated, as Wassermann was able to prove experimentally. However, the circumstances on which these variations depend are as yet entirely unknown to us. Possibly we are dealing here with subtle pathological changes.

Source of the Complements — Leucocytes as a Source — Other Sources. — *Where do the complements or alexins originate?* This question has been studied particularly by Metchnikoff and by Buchner; also by Bail, Hahn, Schattenfroh, and others. These investigators believe that the leucocytes are the source of the complements or alexins. There is, however, this difference between the views of Metchnikoff and Buchner: whereas Buchner believes the alexins to be true secretory products, Metchnikoff believes that they originate on the breaking up of the leucocytes, i.e., that they are decomposition products. Metchnikoff bases his belief chiefly on the work of his pupil, Gengou, who showed that although the serum was rich in alexin (i.e., complement) the plasma contained none at all.

Other authors, as Pfeiffer and Moxter, as a result of their experiments, are not willing to assume the existence of any relationship between the alexins and the leucocytes. Gruber as well as Schattenfroh are ready to believe the leucocytes to be the

source of *an* alexin, but claim that this is different from that found in serum. Wassermann believes that the leucocytes are a source of complements (alexins), for he succeeded in producing anticomplement by means of injections of pure leucocytes which had been washed free from all traces of serum, and which had been obtained by injections of aleuronat. In view of the plurality of the complements, Wassermann expressed the view that the leucocytes are probably *one* source, but not the only one, for the complements of the serum. Landsteiner and Donath have confirmed this experimentally. They succeeded in producing anticomplement by the injection, not only of leucocytes, but of other animal cells. Furthermore, the experiments of Ehrlich and Morgenroth already mentioned, in which the complements disappeared after the destruction of the liver function, show that the liver cells are concerned in the formation of complements.

Structure of Complements — Haptophore and Zymotoxic Groups — Complementoids. — The structure of the complement has been studied particularly by Ehrlich and Morgenroth, and by P. Müller. We have seen that the complements lose their power when heated to 55° C. If, however, we inject animals with a normal serum that has previously been heated to 55° C., we shall still excite in these animals the production of anticomplements. This shows that the heating has not destroyed the entire

complement body, but only that part which affects the digesting, solvent action. The part of the complement concerned with the combination with the inter-body or immune body, in other words, that part called by Ehrlich the haptophore group, must have remained intact. It is clear, therefore, that anticomplements can only be formed when there remain in the complements haptophore groups

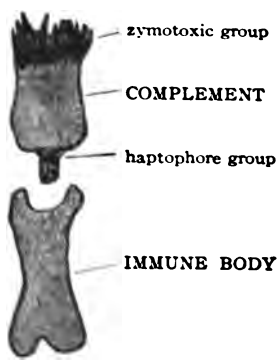


FIG. 9.

that fit certain receptors in the organism of the animal injected. From this it follows that the complements consist of a combining *haptophore group* which withstands heating to 55° C., and another more fragile group which possesses the actual solvent properties, and which Ehrlich calls the *zymotoxic group*. There is a perfect analogy between this and the toxins already studied. These, it will be remembered, consist of a haptophore and

a toxophore group. And just as those toxins which had lost their toxophore group were called toxoids, so Ehrlich and Morgenroth purpose to call complements which have lost their zymotoxic group, *complementoids*.

Isolysins — Autolysins — Anti-isolysins. — All of the preceding studies in hæmolysis have concerned themselves with the results obtained by injecting animals of one species with blood cells of another. Ehrlich and Morgenroth now sought to discover what the results would be if they injected an animal with blood cells of its own species. They injected goats with goat blood, and found that when the amount injected at one time was large the serum of the goat injected acquired hæmolytic properties for the blood of many other goats, but not for all. The substances thus formed the authors called *isolysins*. These, then, are substances which will dissolve the blood of other individuals of the same species. Substances which dissolve the blood cells of the same individual are called *autolysins*. But autolysins have so far been demonstrated experimentally only once (by Ehrlich and Morgenroth). If one tests the properties of an isolysin of a goat on the blood of a great many other goats, it will be found that this will be strongly solvent for the blood of some, slightly for the blood of others, and not at all for still others.

By using a blood that was readily dissolved by

the isolysin, and proceeding in the same series of experiments which we have already studied under hæmolysis, Ehrlich and Morgenroth showed that the isolysins, like the hæmolysins, consist of an immune body and a complement of the normal serum. The experiments undertaken by these authors were made on thirteen goats, and the surprising fact developed that the thirteen resulting isolysins were all different. For example, the isohæmolytic serum of one goat dissolved the red cells of goats *A* and *B*; that of a second goat those of *C* and *D*; of a third those of *A* and *D*, but not of *C*, and so on. If now they produced *antiisolysins* by injecting animals with these isolysins, they found that these antiisolysins were specific; i.e., the antiisolysin of *A* would inhibit the action only of isolysin of *A*, but not of *C*, etc. These results are of the highest clinical interest, for they show a *difference in similar cells of the same species*, something that had never before been suspected. In the above, the blood cells of species *A* must have a different biological constitution than those of species *C*, etc.

The fact that after injections of large amounts of cells of the same species isolysins develop, but that autolysins are almost never formed, caused Ehrlich and Morgenroth to assume that the body possesses distinct regulating functions which naturally prevent the formation of the highly destructive

autolytic substance. It is obvious that if there were no such regulating facilities, the absorption of large bloody effusions and hemorrhages might lead to the formation by the organism of autolysins against its own blood cells. Gengou, a pupil of Metchnikoff, believes to have shown experimentally that the destructive action of these autolysins is hindered by the simultaneous production of an auto-antiimmune body which immediately inhibits their action.

In order that isolysins may be formed, it seems necessary to overwhelm the organism once or several times with large amounts of cells or cell products of the same species; to produce, as Ehrlich says, an *ictus immunisatorius*. Wassermann tried, by using various blood poisons, such as hæmolytic sera, toluylenediamine, etc., for a continued length of time, to cause the formation of these isolysins, but without success, although in these experiments each injection was followed by an appreciable destruction of red cells and absorption of their decomposition products. The gradual and even repeated absorption of not too large quantities of decomposed red cells does not therefore lead to the formation of isolysins; but, as already said, a sudden overwhelming of the organism by large amounts of the cells or their products is necessary.

Deflection of Complement. — In the use of the antitoxic sera, experience has shown that the em-

ployment of a large dose is of paramount importance. So far as the antitoxic action is concerned [1] one cannot do harm by giving a large excess. Concerning the action of bactericidal sera, however, the literature contains a number of examples which indicate that here an excess of immune serum is occasionally injurious. Perhaps the earliest protocol of this kind is that published by Löffler and Abel [2] on their experiments with bacillus coli and a corresponding immune serum. Out of nineteen guinea pigs which had been inoculated with the same amount of culture and had received varying amounts of immune serum, only six animals were protected, those which had received doses of 0.25 c.c. to 0.02 c.c. Eight animals with larger doses, as well as five with smaller doses of serum died. Neisser and Wechsberg [3] encountered the same phenomenon in bactericidal *test-tube* experiments, and concluded as a result of their experiments that the only satisfactory explanation was one based on the views of Ehrlich and Morgenroth. In Fig. 10, *A* II represents schematically a bacterium *a* with a number of receptors; for there are many reasons for assuming that each bacterium possesses a

[1] We shall discuss the rash production, or "serum sickness," page 138.

[2] F. Löffler and R. Abel, Centralblatt Bacteriol., 1896, Vol. xix, p. 51.

[3] M. Neisser and F. Wechsberg, Münch, med. Wochenschrift, 1901. No. 18.

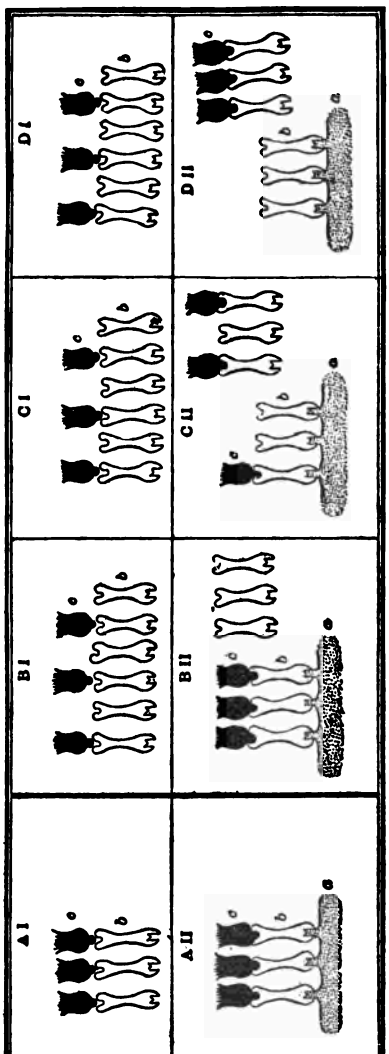


FIG. 10

number of receptors of the same kind. According to the side-chain theory, if we inject this bacterium into an animal an over-production of the corresponding group will occur, resulting in a serum which is rich in body *b*. This body *b*, however, is not able by itself to injure the bacteria, and a bacterium all of whose receptors are laden with *b* need not at all be injured in its vitality. Body *b* normally possesses a peculiar function, namely, to serve as a coupling member or link, and hence it possesses two groups (amboceptor). As has already been discussed, one of these groups fit the receptors of the bacterium on the one hand and the complement on the other. When, therefore, to a normal serum which contains suitable complement, we add equivalent amounts of immune serum, the condition pictured in *A* I will result. On adding the corresponding bacterium to this we get the condition shown in *A* II, in which all the bacterial receptors are occupied with immune bodies, or more accurately, with immune bodies which on their part are loaded with bacteriolytic complement *c*. In the case here presented let us say that it requires the occupation of all of the receptors with complemented interbodies to cause the death of the bacterium.

If now to an equivalent mixture of complement and inter-body we add an excess of inter-body, it will be possible for only a *part* of the inter-body to

be loaded with complement, leaving a portion of the inter-body uncomplemented. On adding the corresponding bacteria a number of conditions may result; the affinity of the inter-body for the bacterial receptor may, as a result of the loading with complement, (1) remain *unchanged*, (2) it may thereby be *increased*, or (3) be *diminished*.

In the figure, *B* II shows the condition of increased affinity. Of the six inter-bodies only those combine with the bacterium which have become laden with complement. In this case, therefore, the excess of inter-bodies will have no influence on the bactericidal effect. The condition is really the same as *A* II, except that free inter-body is also present.

C II shows the condition of unchanged affinity. In this case, if we add the bacterium to the mixture of complement and excess of inter-body, all the receptors of the bacterium will, to be sure, be occupied by inter-bodies, but this will be entirely without regard to the fact that these inter-bodies are or are not loaded with complement. It may therefore happen that only a few of the bacterial receptors will be occupied by complemented (i.e., active) inter-bodies, while the rest of the bacterial receptors are occupied by uncomplemented (hence inactive) inter-bodies. As already stated, however, the vitality of such a bacterium is not necessarily destroyed.

D II represents the last conceivable case. It is assumed that the " completion " of the inter-body has resulted in a diminution of the latter's affinity for the bacterial receptor. In this case primarily only the uncomplemented inter-bodies will combine with the bacterial receptors, while the free fluid will contain complemented inter-bodies.

In cases *C* II and *D* II, therefore, the *excess of inter-body exerts a deflecting action on the complement*, thus diminishing the end results.

It is difficult to say to what extent " deflection of complement " really occurs in the experiments referred to above. Recent studies by Buxton [1] and others show that deflection of complement will not always explain the phenomenon, and that in these instances other factors must be responsible for the paradoxical results.

For the absorption of complement commonly known as the " Bordet-Gengou," or the " Gengou-Moreschi " phenomenon, see page 68. To avoid confusion it will be well to restrict the term " deflection of complement " to the phenomenon described by Neisser and Wechsberg.

Deutsch's Hæmolytic Blood Test. — Deutsch [2] in 1900 suggested the use of artificial hæmolysins in legal medicine, in the identification of bloods,

[1] Buxton; Journal Medical Research, Vol. xiii, 1905.

[2] Deutsch, Die forensische Serumdiagnose des Blutes, Centralblatt Bacteriol., Vol. xxix, 1901.

both fresh and dried. He found that a powerful hæmolytic serum dissolved powdered blood completely, the latter being suspended in 0.9% salt solution. Dried blood to which saline is added brings the hæmoglobin of the injured corpuscles into solution, the uninjured corpuscles do not, however, dissolve even after twenty-four hours at 37° C. If the dried blood is extracted in normal rabbit serum, more hæmoglobin goes into solution than with saline, when the proportion added is 1:2, whereas the normal serum acts like saline when added in the proportion of 1:4. When two samples of the same dry blood are brought into suspension in normal and artificial hæmolytic serum, respectively, a little phenol or toluol being added, the antiserum brings about complete hæmolysis after twenty-four hours, besides leading to the formation of a precipitum, due to the action of precipitins formed in the blood-treated animal in consequence of the serum which was injected together with the corpuscles. When washed corpuscles alone are injected precipitins are not formed. In view of the specificity of the reactions observed with human blood, Deutsch considers that the method can be put to use in a practical way. There can, however, be no question but that the precipitins offer many advantages over the hæmolysins for such purposes.

For other biological blood tests see the Wasser-

mann-Uhlenhuth precipitin test, page 112, and the recent Neisser-Sachs test, page 70.

Practical Value of Injections of Bactericidal Sera. — The use of sera having specific protective properties has been tried practically on a large scale in man as a preventive of infection. It is difficult to estimate just what value these injections have had. In susceptible animals, injections of some of the very virulent bacteria, as pneumococci, streptococci, typhoid bacilli, and cholera spirilla, can be robbed of all danger if small doses of their respective serums are given before the bacteria have increased to any great extent in the body. If given later they are ineffective. For some bacteria, such as tubercle bacilli, no serum has been obtained of sufficient power to surely prevent infection. Through bactericidal serums, therefore, we can immunize against an infection, and even stop one just commencing; but as yet we cannot cure an infection which is already fully developed, though even here there is reason to believe that we may possibly prevent an invasion of the general system from a diseased organ, as by the pneumococcus from an infected lung in pneumonia. On the whole, the bactericidal sera have not given, as observed in practice, conclusive evidence of great value in already developed disease.

It is apparent from all that has been said that a deeper insight into the mechanism of the bacteri-

cidal sera has disclosed many difficulties to be overcome before we can hope for much in a practical way. Thus we have as yet found no method of increasing the complements, and these are apparently highly important in destroying the invading bacteria. Nor have we any way to determine the proper dose so as to avoid the phenomenon termed " deflection of complement." Furthermore, we now know that the defence of the animal body against bacterial invasion is not solely a matter of bactericidal and antitoxic substances. The brilliant studies of Ehrlich, Bordet, and others on the *humoral* side of immunity has until recently caused the *cellular* side advocated by Metchnikoff to be much neglected. Perhaps the recent work begun by A. E. Wright on opsonins may lead us in the right direction. The therapeutic results thus far achieved by the use of bactericidal immune sera certainly show that much remains to be done in the study of immunity.

PRECIPITINS

Definition. — All of the foregoing experiments have concerned themselves with the results obtained by injection of cellular material of one animal into another. In the further study of this subject, experiments were made to discover what happens when *dissolved* albuminous bodies of one species are injected into animals of another species. This line of investigation was first pursued by Tchistowitsch,[1] who injected rabbits with the serum of horses and of eels. On withdrawing serum from such rabbits and mixing it with horse or eel serum, the mixture became cloudy, owing to the precipitation of part of the albumin of the horse or eel serum by that of the rabbit. Normal rabbit serum does not possess this property. Bordet was able to demonstrate that the same thing takes place if rabbits are treated with chicken blood. On mixing such a serum with chicken serum, a precipitate formed. The substances which develop in the serum by treating an animal with albuminous bodies of another animal, and which precipitate these albumins when the sera of the two animals are mixed, are

[1] Tchistowitsch, Annal. Pasteur. Vol. xiii, 1899.

called *precipitins*.[1] This power of the organism to react to the injection of foreign dissolved albuminous substances has been found to be very extensive.

Bacterial Precipitins. — In 1897, R. Kraus showed that the serum of a rabbit immunized against typhoid often produces a precipitate in the bacterial-free filtrate of a bouillon culture of typhoid bacilli. This fact has been verified by subsequent investigators and the reaction found to be specific. In general, the best results are obtained with old bouillon cultures which contain a larger proportion of the autolytic products. It was natural that this reaction should at once be applied to the diagnosis of typhoid and other diseases. Numerous experiments however have shown that Kraus' phenomenon is not nearly so constantly observed as that of agglutination, and the reaction is therefore but little used. Whether the bacterial precipitins are identical in character with those obtained by injecting an animal with an unrelated serum (zoöprecipitins), is still undecided. Rostoski, as well as Nuttall, believes that they are probably different. So much for bacterial precipitins.

Lactoserum — Other Specific Precipitins. — Bordet, by injecting cows' milk into rabbits', was able to produce a serum which precipitates the casein of

[1] It will be recalled that, besides the production of precipitins, the above procedure causes the formation of other anti-bodies such as anti-complements, anti-agglutinins, etc.

cows' milk. He called this *lactoserum*. Ehrlich, Morgenroth, Wassermann, Schütze, Myers, and Uhlenhuth showed that by treating a rabbit with chicken albumin a precipitin is formed which precipitates chicken albumin. Myers, by treating animals with Witte's pepton and globulin, produced a serum that contained specific antipeptons and antiglobulins. Pick and Spiro, by using albumose, produced antialbumoses. Leclainche and Vallée, Stern, Mertens, and Zülzer treated animals with human albuminous urine and produced a serum which contained a precipitin specific for this substance. Schütze, by treating rabbits with a vegetable albumin, as well as with human myoalbumin, produced a precipitin specific for these albumins. This does not exhaust the recital of the work done in this field, and there is a host of other albuminous bodies which, when injected into an animal, are able to excite the production of precipitins.

Specificity of the Precipitins. — It was soon recognized that the specificity is not absolute. Above all, this depends upon the strength of the serum, i.e., its degree of activity. This is measured by the dilution in which it will still react. Thus a highly active serum, one, for example, which will still give a distinct reaction when diluted 1 : 1000 or over, will produce a marked precipitate with the serum used to excite its production; whereas, in the serum of other animal species it will produce slighter pre-

cipitates, or only cloudings. A less highly active serum will likewise cause a marked precipitate in the homologous blood solution, and a slight precipitate, or only a clouding, at the most, in a closely related species. For example, the serum of a rabbit which has been treated with sheep blood produces a marked precipitate in a solution of sheep blood; a slight precipitate in a goat-blood solution; and a still fainter one in an ox-blood solution. In some instances the two latter will show only a clouding. If we employ a very weak serum, even the cloudings will be absent, and a precipitate is formed only in the sheep-blood solution. If human blood or blood serum has been injected, the clouding and precipitation will occur most readily (aside, of course, from human-blood solution) in that of apes. In the precipitin reaction, therefore, the relationship of the single animal species is an important factor. This peculiar behavior has first been thoroughly studied by Nuttall [1] who made observations on five hundred different animals. As a result of these we know that a weak human-blood antiserum, besides reacting on human blood, causes a clouding only in the blood of anthropoid apes (chimpanzee, gorilla, orang-outang); a stronger serum causes a clouding also in the blood of other monkeys; finally

[1] *British Medical Journal*, 1901, Vol. ii, and 1902, Vol. i. See also Nuttall, Blood Immunity and Blood Relationship, 1904. The Macmillan Co., N. Y.

a very highly active serum reacts with the blood of all the mammalia. In that case, of course, only a faint clouding is produced even after considerable time. Nuttall also obtained antisèra, each of which was specific for one of the large animal classes (birds, reptiles, amphibia). Here, too, the same quantitative differences were noted.

Nature of the Precipitins. — The precipitins are fairly resistant bodies, whose power gradually declines at a temperature of 60° C., but is not lost until 70° C. is reached. Once their action is lost, it cannot be restored by the addition of normal sera, showing that the precipitins, like the agglutinins, are receptors of the second order and are not amboceptors. The resulting precipitate is soluble in weak acids and alkalies. Peptic digestion destroys the substances which effect the precipitation. Leblanc found that the precipitins were precipitated from the serum in that fraction which Hofmeister calls the *pseudo globulins*. Eisenberg, on the other hand, in his experiments found them in the *euglobulin* fraction. The latter result was also obtained by Obermayer and Pick in precipitins obtained from goats and rabbits. The discordant results are comprehensible in view of recent publications concerning the unreliability of ammonium sulphate fractionation of serum globulins. The nature of the resulting precipitate has also been studied by Leblanc. He finds that it is a combination of the

precipitated albumin with the antibody of the specific serum. In this combination the properties of the *pseudo* globulin predominate showing that it is the specific serum which furnishes the greater part of the precipitate. The presence of salts seems to be necessary for the precipitin reaction. A temperature of 37° C. hastens, while a low temperature markedly retards the reaction. In either case, the amount of precipitum is uninfluenced. The presence of even small quantities of acids or alkalies markedly reduces the amount of precipitum formed, but an increase of salt (NaCl) has little effect.

Practical Application. — These precipitins have very recently found a practical application. Fish, Ehrlich, Morgenroth, Wassermann, and Schütze investigated the specific action of lactoserum. They found that a serum derived by treating an animal with cows' milk contained a precipitin which reacted only on the casein of cows' milk, but not on that of human milk or goats' milk. The serum of an animal treated with human milk was specific for the casein of human milk, etc. Ehrlich, Morgenroth, and Wassermann also experimented with the serum resulting from treatment with chicken egg albumin, and found that this, while not strictly specific so far as closely related species are concerned, is yet so against other species. *The precipitins, therefore, react on closely related albumins, but are specific against those of unrelated species.*

The Wassermann-Uhlenhuth Blood Test. — As a result of these researches Wassermann proposed, at the Congress for Internal Medicine, 1900, to use these sera as a means of differentiating albumins, i.e., to distinguish the different albumins from one another, and particularly to distinguish those derived from man from those of other animals. This proposal thus to use the Tchistowitsch-Bordet precipitins had important practical and theoretical results. Uhlenhuth, Wassermann, Schütze, Stern, Dieudonné, and others showed that a serum could be produced from rabbits by injecting them with human serum, by means of which it is possible to tell positively whether a given old, dried blood stain is human blood or not.

Uhlenhuth [1] tested nineteen kinds of blood and only obtained a reaction with human blood upon adding antihuman serum to the series of dilutions. He, moreover, found that human blood which had been dried four weeks on a board could be readily distinguished by means of antihuman serum from the blood of the horse and ox. On the following day Wassermann [2] demonstrated experiments similar to Uhlenhuth's at the meeting of the Physiological Society, Berlin. Outside of human blood only that of a monkey gave the reaction with antihuman serum.

[1] Uhlenhuth, Deutsche med. Wochenschrift, 1901. xxvii.

[2] Wassermann A. and Schütze, Berliner klin. Wochenschr. 1901. No. xxviii.

The reliability of this reaction in medico-legal questions has been abundantly established. In the forensic blood diagnosis the subjects of the test are usually blood stains on clothing, and on wood and metal objects. After such a doubtful stain has been dissolved in physiological salt solution, one first proceeds to determine that it is really blood. For this purpose Teichmann's test (the production of hæmin crystals), the guaiac test, and the spectroscopic examination are undertaken. This is of considerable importance, for not merely blood but other albuminous solutions derived from the same animal react with an antiserum obtained by injecting an animal with blood or serum. Having found that the stain is that of blood, we next determine the special kind of blood.

Immunizing the Animals. — For the production of the antisera, we make use of rabbits. These can be injected either with sterile, freshly-defibrinated blood or with sterile serum, the latter being preferable for intravenous inoculation. It is well to begin with small doses and gradually increase; thus for intravenous inoculations the first injection should be about one c.c. and increased up to three or four c.c. With intraperitoneal injections about double these doses can be given. Ordinarily, the interval between injections is three or four days, and the entire duration of treatment from two weeks to a month. Long-continued treatment

leads to a disappearance of precipitins from the blood.

Collecting the Serum. — When the animals have received five to six injections, and some days have elapsed it is well to draw off samples of the blood and to test for precipitins. This is easily done by shaving the ear and cleansing the skin with alcohol and sterile water. An incision is then made into the marginal vein and a few drops of blood collected in a small test-tube. This is then set aside to allow the blood to coagulate. After the serum has separated it can be tested and if it prove insufficiently powerful, treatment may be continued, otherwise the animal may be killed, preferably a week or ten days after the last injection. The animals may be killed in a variety of ways. Uhlenhuth chloroforms them, opens the thoracic cavity under aseptic precautions, and, cutting through the beating heart, the blood is allowed to flow into the thoracic cavity, whence it is removed by means of sterile pipettes to suitable vessels. Nuttall's method is to shave the neck and disinfect the skin with lysol solution; bend the animal's head backward to put the skin of the neck on the stretch, and have an assistant make a clean sweep with a sterilized knife through the tense skin to and through the vessels. The blood spurts into a large sterile dish which is immediately covered when the main flow has ceased. The dishes are placed horizontally until a clot has

formed; they are then slightly tilted, and as soon as serum enough has been expressed, this is pipetted off into sterile test containers which are stored in a cool place. It is well not to add any preservative to the serum, as such an addition may occasionally lead to *pseudo* reactions.

The Test. — In carrying out the test the suspected clot is mixed with a small quantity of normal salt solution and then filtered. Whether or not the blood specimen has gone into solution can best be judged by the *foam test.* Air is blown gently through the pipette which is used for transferring the solution into the test-tubes. Solutions of blood or serum of 1 : 1000 and over, still foam well. The color of the fluid is not so reliable an index of solution. To some of this solution in a test-tube, about double the amount of the specific serum (derived as above) is added. As a control test, we place a little blood of another species, e.g., of an ox, in a second test-tube together with some of the specific serum and a little normal salt solution. In a third tube we place some of the suspected blood solution, and in a fourth some of the specific serum mixed with the normal salt solution. All four tubes are placed in the incubator at 37° C. for one hour, or are left at room temperature for several hours. If the suspected clot was one of human blood, the first tube will show distinct evidence of precipitation, while all the control tubes will have remained clear. It

is desirable to dilute the suspected blood as far as possible when testing, for when concentrated sera are brought together reactions may occur which will lead to erroneous conclusions. In medico-legal work it will be well to progressively dilute a suspected blood sample and to reach a conclusion upon the highest (within limits) which reacts to a given antiserum. In routine work one can commence with dilutions of the suspected blood of 1:100 or 1:200. We must not omit to say that it is necessary to test to litmus all solutions to be examined, and to neutralize any that are found decidedly acid or alkaline.

Appearance of the Reaction. — When antiserum is added to blood dilution it sinks to the bottom of the tube, forming a milky white zone at the point of contact. The milkiness gradually extends upward until the whole fluid is clouded. Where the fluids have been mixed by shaking this diffuse cloudiness undergoes a change; after ten to twenty minutes, or later, very fine granules of precipitum begin to appear, and the upper layers of the fluid begin to clear, due to sedimentation of the precipitum. The fine particles soon become aggregated into coarser ones, and these into flocculi which, gradually sinking to the bottom of the tube, give rise to more or less deposit of a whitish appearance. With blood dilutions of, say 1:40 to 1:200 and over, the deposit formed is usually sharply defined; where more con-

centrated dilutions are used, the deposit may form an irregular mass at the bottom of the tube.

The reaction may be followed microscopically by means of the hanging-drop method. By this method a reaction can be observed within ten to fifteen minutes, which macroscopically becomes visible only after two hours.

Delicacy of the Precipitin Test. — Whereas the ordinary chemical tests cease to give reactions in blood dilutions of about 1 : 1000, powerful antisera greatly exceed this limit, as the reported results of independent observers have shown. Working with an antihuman serum, Strube reports a reaction with a blood diluted 20,000 times, and Stern one with a blood diluted 50,000 times. Ascoli obtained a reaction with a specific serum with egg albumin diluted 1,000,000 times.

Other Applications of the Precipitin Test. — It can be readily understood that this test finds ready application in the detection of horse, dog, or cat meat in sausage.

The principle and the method are the same in all these various applications. We treat animals with the albumins which we wish to differentiate, and so obtain sera specific, each for its particular kind of albumin. These sera, then, produce precipitates only in solutions of their respective albumins. For example, if we wish to determine whether a given sample of meat is horse-flesh or not we must inject

an animal with horse serum, or, if we prefer, with an extract of horse-flesh. The serum derived from this animal will then produce a precipitate in the aqueous extract of the meat if this be horse-flesh, but not if it be beef. Animals treated with dog serum yield a serum which precipitates an aqueous extract of dog-flesh, etc. The method of examination consists in scraping the meat and extracting it with water or normal salt solution. It takes a long time to extract the meat in some cases. An extract is suitable for testing when it foams on being shaken. If the extract is very cloudy it should be cleared by filtration through a Berkfeld filter. In testing, add ten to fifteen drops of antiserum to 3 cc. of the saline meat extract.

Antiprecipitins — Iso-precipitins. — Biologically, the precipitins are found to behave like the substances already studied. It is possible, for example, by injecting an animal with a precipitin, say lactoserum, to obtain an *antiprecipitin*, an anti-lactoserum, which counteracts or inhibits the action of the precipitin. This is entirely analogous to the antihæmolysins, the antispermotoxin, etc.

If rabbits are treated with rabbit serum, a serum is obtained which will, in certain cases, precipitate the serum of other rabbits. This was done by Schütze, and he called this serum *iso-precipitin.*

II. CYTOTOXINS

Cytotoxins — Definition — Leucotoxin — Nature of the Cytotoxin — Anticytotoxin. — After it had been found that the injection of an animal with red blood cells of another animal was followed by the production of definite, specific reaction substances, investigators experimented to see whether this was also the case if other animal cells were used. Injections were made with white blood cells, spermatozoa of other animals, etc., and there resulted a series of reaction substances, entirely analogous to the hæmolysins, which were specific for the cells used for injection. These sera Metchnikoff calls *cytotoxins*. After Delezenne had published a short article on a serum hæmolytic for white blood cells, Metchnikoff undertook a study of the substances produced in sera of animals treated with leucocytes of another species. He injected guinea pigs with the mesenteric glands and bone marrow of a rabbit. He also injected for several weeks half an Aselli's pancreas at a time, at intervals of four days. If he withdrew serum from such a guinea pig he found this to be intensely solvent for white blood cells of a rabbit. He called this serum *leucotoxin*. This leucotoxin is very poisonous for these animals, and

kills them within a few hours. Non-fatal doses at first excite a marked hypoleucocytosis, which is followed after a few days by a compensatory hyperleucocytosis. Leucotoxin destroys the mononuclear as well as the polynuclear leucocytes of the animal, as was shown by Funk. Leucotoxin which had been derived by injection of the leucocytes of horses, oxen, sheep, goats, or dogs acted only on the leucocytes of that species, not on the leucocytes of man. So far as the mechanism of the cytotoxic action is concerned, it has been found that this is the same as that of the hæmolysins. The action of the specific cytotoxic serum is always due to the combined action of two substances in the serum, a specific immune body, and an alexin or complement present also in normal serum. The cytotoxic sera, like the hæmolytic sera, are rendered inactive by heating to 55° C. In other respects also the cytotoxic sera maintain the analogy to the hæmolytic sera. Thus it is possible by immunizing with a cytotoxin to obtain an *anticytotoxin.* Metchnikoff, for example, was able to produce an antileucotoxin by injecting animals with leucotoxin. This antibody inhibited the action of the leucotoxin.

Neurotoxin. — Delezenne and Madame Metchnikoff have injected animals with central-nervous-system substance, and so produced a specific *neurotoxin.* They injected ducks intraperitoneally, giving

them five or six injections of ten to twenty grammes of dog brain and spinal cord mixed with normal salt solution. The serum of these ducks injected intracerebrally into dogs in doses of 0.5 c.c. caused the dogs to die almost at once in complete paralysis, whereas if normal duck serum was injected in the same way no effects of any kind were produced. If smaller doses of the specific neurotoxic serum were administered, say 0.1 to 0.2 c.c., various paralyses and epileptiform convulsions set in, from which the animals sometimes recovered. The action of this serum is specific, i.e., the serum of ducks treated with dog brain causes these symptoms only in dogs, while on rabbits it acts no differently than normal duck serum.

Spermatoxin. — Another specific cell-dissolving serum was produced by Landsteiner, Metchnikoff, and Moxter, by injecting animals with the spermatozoa of other animals. Such a serum rapidly destroys the spermatozoa of the animals whose product was injected. This cytotoxin was named *spermatoxin*. If animals are treated with spermatozoa there is produced a serum which is not only a spermatoxin, but which is also hæmolytic for the red cells of that animal. This was demonstrated by Metchnikoff and Moxter, and has already been referred to in discussing hæmolysins. If, for example, we inject the spermatozoa of sheep into rabbits, we shall obtain a serum that is sperma-

toxic for sheep, as well as hæmolytic for sheep red cells.

Common Receptors. — At first it was thought that the hæmolysin so produced was due to the presence of small quantities of blood injected with the spermatozoa. The same result however was obtained when all traces of blood could be excluded;[1] furthermore a number of investigators produced hæmolysins by the injection of fluids entirely free from red corpuscles, such as serum and urine. The production of this hæmolysin is not hard to explain if we hold fast to the side-chain theory. We have merely to assume that the spermatozoa or these other substances possess certain receptors in common with the red blood cells of the same animal. Ehrlich and Morgenroth[2] have repeatedly pointed out that *specificity is a matter not of cells, but of receptors.* Despite these very conclusive demonstrations later investigators, who attempted to produce antisera for the cells of various organs, continued to use emulsions of unwashed organs, in utter disregard of the presence of free receptors in the organ juices and also without consideration of the antibodies certain to be produced by the red cells normally present.

Cytotoxin for Epithelium. — As far back as 1899,

[1] Von Dungern. See Collected Studies on Immunity, p. 47. Wiley and Sons, New York, 1906.

[2] Ehrlich and Morgenroth, *Ibid.*, p. 109.

von Dungern showed that it was possible to produce an *antiepithelial* serum by treating animals with the ciliated tracheal epithelium of oxen. This serum was rapidly destructive for this particular kind of epithelium, but it contained also a specific hæmolytic body just as was the case in the spermotoxic serum, and for the same reasons. This antiepithelial serum aroused considerable interest since it indicated the possibility of producing sera which were cytotoxic for certain varieties of epithelial cells, especially those of pathological origin, as carcinoma. The numerous experiments made in this direction failed however to produce the desired results. Owing to the extensive distribution of common receptors the antisera were found to exhibit quite general properties and to lack that degree of cell specificity, essential for practical purposes.

Cytotoxins by the Use of Nucleo-Proteids. — In order to prevent the adventitious formation of those bodies resulting from impure methods of immunization, and also in the hope of obtaining greater specificity, a few investigators have utilized the nucleo-proteids of the cell for immunization. This method seems to have been tried first by Marrassini in 1903, but with indifferent results. In 1905 Beebe[1] published an extensive study along

[1] S. P. Beebe, Cytotoxic Serum Produced by the Injection of Nucleo-Proteids, Journ. Exper. Medicine, Vol vii, 1905.

these lines and described the formation of a nephrotoxic serum which caused albuminuria and acute degeneration of the kidney without changes in the other organs. Albuminuria appeared generally on the fourth or fifth day, increased rapidly in amount, and was accompanied by the excretion of hyaline and granular casts. Recently Pearce and Jackson,[1] after a careful experimental study on the production of cytotoxic sera by the injection of nucleo-proteids, conclude " that the results do not support the theory that specific cytotoxic sera may be developed in this way, but indicate, rather, that such sera have certain mildly toxic properties acting in a general way and affecting especially the principal excretory organ, the kidney."

[1] R. M. Pearce and Holmes Jackson, Journal of Infectious Diseases, Vol. iii, 1906.

OPSONINS OR BACTERIOTROPIC SUBSTANCES

Historical. — The early work of Nuttall and others on the bactericidal action of normal serum, and Pfeiffer's demonstration of the bacteriolysis of cholera and typhoid bacilli by immune sera in the absence of cells, formed the chief basis on which rested the *humoral theory*, which attributed the protection in such cases to the destructive action of the serum on the microbes. It was found, however, that cases of protection resulting from the use of immune serum occurred where no such bacteriolytic action could be demonstrated; infection with plague or streptococcus may be mentioned as examples. It is now pretty generally accepted that immunity in these cases is due largely to the *phagocytic* action of the leucocytes. As far back as 1858 Haeckel had observed that particles of indigo injected into the veins of certain molluscs could shortly afterwards be found in the blood cells of the animal. However, the significance of this and other observations was not appreciated until Metchnikoff [1] in 1883 called attention to their bearing on infection and immunity. The outcome

[1] Arbeiten des Zoölog. Institutes in Wien, 1883, Vol. v.

of his investigations was the establishment of the well-known doctrine of *phagocytosis*, the principle of which is that the wandering cells of the animal organism, the leucocytes, possess the property of taking up, rendering inert, and digesting micro-organisms which they may encounter in the tissues. Metchnikoff believes that susceptibility to or immunity from infection is essentially a matter between the invading bacteria on the one hand and the leucocytes of the tissues on the other. He realizes that the serum constituents play an important rôle, but this rôle consists in their *stimulating the leucocyte* to take up the bacteria.

Thus if a highly virulent organism is injected into a susceptible animal, the leucocytes appear to be repelled, and to be unable to deal with the microbe, which multiplies and causes the death of the animal. If, however, the suitable immune serum is injected into the animal before inoculation, the phagocytes attack and devour the invading micro-organisms. Admitting that the phagocyte plays an important part in certain infections the question must still be considered whether the immune serum has acted on the injected microbes or on the phagocytes. Metchnikoff, we have seen, takes the latter view.

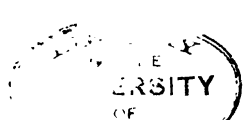
In 1903 A. E. Wright [1] called attention to certain substances present in serum which acted on bacteria

[1] Wright and Douglas, Proc. Royal Society, Vol. 72, 1903.

and rendered them more easily taken up by the phagocytic cells. He called this substance *opsonin* and showed that it is present in normal as well as immune sera. By means of absorption tests modelled after those of Ehrlich and Morgenroth, he showed that the opsonin has a specific affinity for the bacteria and none for the leucocytes. The opsonins for staphylococcus prepare only staphylococci for the leucocytes, those for tubercle bacilli only these bacteria, etc. As a result of his observations Wright supposes that the phagocytes play only a passive rôle, which depends on the preliminary action of the opsonin.

Bacteriotropic Substances. — Independently of Wright, though somewhat later, Neufeld and Rimpau [1] of Berlin published experiments on the phagocytic effect of immune sera. They also found that in these sera there exists a substance which has no direct action on the phagocytes, but which can fix itself on the corresponding bacteria and so modify these that they are more readily devoured by the phagocytes. They call this constituent a "bacteriotropic substance." There is little doubt that this bacteriotropic substance and Wright's opsonin are identical. Certain differences in the effect of heat are probably to be explained by the differences in the quantities of these sensitizing substances in normal and immune sera.

[1] Neufeld and Rimpau, Deutsche med. Wochenschrift, 1904.



Opsonins Distinct Antibodies. — It was natural to question whether these " opsonins " were really distinct from other antibodies, or whether they were perhaps identical with the immune body (or substance sensibilatrice). In a series of papers on this subject Hektoen [1] shows that the former is the case, opsonins are distinct substances. This is not only indicated by the results of absorption tests, but by the fact that, by immunization, a serum can in certain cases be obtained which is opsonic but not lytic, or in other cases one which is lytic but not opsonic. Similar experiments have differentiated opsonins from agglutinins.

Structure of Opsonins. — Opsonins, like agglutinins and precipitins, appear to possess two groups, opsoniferous and haptophore. On heating an opsonic serum the former group is destroyed but the haptophore group remains intact, as can be seen from suitable combining experiments. There is still considerable difference of opinion as to the degree of heat necessary to inactivate the opsonins. Once the opsoniferous group has been destroyed it is impossible to restore the opsonic action by the addition of a complementing substance. Hence the opsonins are to be regarded as receptors of the second order and similar in structure to the agglutinins and precipitins.

The Opsonic Index. — In the study of these opso-

[1] Hektoen, L., Journal Infect. Diseases, 1905 and 1906.

nins Wright developed the idea that they were highly important in combating a number of bacterial infections, such as staphylococcus and tubercle. His observations showed that inoculations of the corresponding bacteria produced marked changes in the opsonic contents of the infected individual and that it was possible to estimate accurately the immunizing effect of such inoculations.

Technique. — Wright's technique of measuring the opsonic power is a slight modification of the Leishman[1] method and is as follows: An emulsion of fresh human leucocytes is made by dropping twenty drops of blood from a finger prick into 20 c.c. normal salt solution containing one per cent sodium citrate. The mixture is centrifuged, the supernatant clear fluid removed and the upper layers of the sedimented blood cells transferred by means of a fine pipette to 10 c.c. normal salt solution. After centrifuging this second mixture the supernatant fluid is pipetted off and the remaining suspension used for the opsonic tests. Such a " leucocyte emulsion, ' of course, contains an enormous number of red blood cells; the proportion of leucocytes, however, is greater than in the original blood.

One volume of this emulsion is mixed with one volume of the bacterial suspension to be tested and with one volume of the serum. This is best accomplished by means of a pipette whose end has been

[1] Leishman, British Medical Journal, Jan., 1902.

drawn out into a capillary tube several inches in length. With a mark made about three-quarters of an inch from the end it is easy to suck up one such volume of each of the fluids, allowing a small air bubble to intervene between each volume. All three are now expelled on a slide and thoroughly mixed by drawing back and forth into the pipette. Then the mixture is sucked into the pipette, the end sealed and the whole put into the incubator at 37° C. The identical test is made using a normal serum in place of the serum to be tested. Both tubes are allowed to incubate fifteen minutes and then examined by means of smear preparations on slides spread and stained in the usual way. The degree of phagocytosis is then determined in each by counting a consecutive series of fifty leucocytes and finding the average number of bacteria ingested per leucocyte. This number for the serum to be tested is divided by the number obtained with the normal serum and the result regarded as the *opsonic index* of the serum in question. The presence of a high opsonic index Wright regards as indicative of increased resistance. He further states that the fluctuation of the opsonic index in normal healthy individuals is not more than from .8 to 1.2, and that an index below .8 is therefore almost diagnostic of the presence of an infection with the organism tested.

Application of the Opsonic Measurements. — At the present time Wright has correlated all his obser-

vations and built up a system of treating bacterial infections by means of active immunization controlled by opsonic measurements. The principles underlying his method may be briefly summarized as follows: In localized bacterial infections the infected body absorbs but small amounts of bacterial substances or antigens. In consequence of this the amount of active immunity developed is but slight. Localized infections therefore tend to run a chronic course. The logical method of effecting a cure in these cases is to actively immunize the body with the invading organism. In a number of infections, notably those of staphylococcus, streptococcus, and tubercle, the degree of immunity is measured accurately by the opsonic index. Following an inoculation with the infecting bacteria (dead cultures in salt solution) there is first a drop in the opsonic index, the " negative phase," then, depending on the size of the dose and the reacting power of the individual, there comes a rise of the index, the " positive phase," or a continuation of the negative phase. The former is obtained with proper dosage; the latter with doses too large or too small. In estimating the size of the dose given, Wright counts the number of bacteria per c.c. of emulsion injected. Thus in the case of localized staphylococcus infections the doses for adult humans range from 100 million to 500 million bacteria. In the case of streptococcus the doses are smaller, averaging about 50

to 100 million. The bacterial suspensions are heated to 60° C. for twenty minutes, 0.5% carbolic acid is added, and tests are made to insure sterility. The time for inoculation is governed by the opsonic index. If the first inoculation has been properly gauged there is a brief negative phase, followed by a positive phase of some days' duration. As this positive phase gradually drops, one gives another inoculation and watches the effect on the opsonic index. If the index drops markedly and rises but little, the dose has been too large. Or if the negative phase is slight, and the positive phase slight and transitory, the dose has been too small. With proper dosage the negative phases are small, and the opsonic index is kept fairly well above normal. Hand in hand with this goes an improvement in the clinical symptoms.

Wright and his pupils have published accounts of a large number of cases successfully treated according to this method. The results are reported as especially good in cases of severe acne, multiple boils, lupus, tubercular glands, and bone tuberculosis.

In judging of the value of Wright's method we must bear clearly in mind that the essential feature of it is the *control by opsonic measurements;* treatment of bacterial infections by the inoculation of dead cultures has long been known.

The results obtained by most workers in this country fail to bear out Wright's claims for the method.

Thus the author[1] finds that the variation in the opsonic indices of several normal persons is often considerable; that opsonic counts based on fifty leucocytes may occasionally vary by more than 50% and that it is therefore necessary to count from 150 to 200 leucocytes for each test; that duplicate, triplicate and more tests made of the same serum, at the same time, and under identical conditions so far as one can tell, frequently give widely divergent results; that the opsonic index and the clinical course of the disease do not always run parallel. Cases may do very well and have the index remain low; other cases may do poorly with an increased opsonic index. It is to be noted, furthermore, that some of these variations in results are unavoidable, at least with the present technique.

To one who has followed the progress of immunity studies, it is not at all surprising to find that the opsonic index is not necessarily a measure of the patient's immunity. When Gruber and Durham published their observations on agglutinins the phenomenon was at once hailed and interpreted by many as measuring the degree of immunity possessed by the patient. The same error was made when some time later the bacteriolytic substances were discovered. In both cases it was soon found that these were but accompaniments of greater or less significance to the complex phenomenon of immun-

[1] Bolduan, Long Island Med. Journal, Vol. i, 1907.

ity. When we consider how manifold are the defensive agencies which the animal organism possesses, and how very complex they become the more they are studied, we shall not marvel at the absence of parallelism between the clinical course of the disease and the opsonic index. There is little doubt that the opsonic indices do measure a certain fraction or phase of the immunity reaction; we do not believe that they replace clinical observations in measuring the effect of immunizing injections.

VII. SNAKE VENOMS AND THEIR ANTISERA

Despite the fact that venomous serpents have excited the fear and interest of mankind for centuries it is only very recently that we have come to know anything definite about their poisons. This is perhaps in part due to the fact that Europe possesses but few poisonous snakes, and so offered little material for study. Some idea of the importance of the subject for certain countries, however, can be seen when it is stated that in India more than 20,000 persons annually die from the bite of the hooded cobra. It was quite natural, therefore, that one of the earliest modern researches into the nature of snake venom, that of Calmette,[1] should have come from that country. This author also found that he could produce an antitoxic serum by injecting animals with the snake venom.

The Venoms. — Our present knowledge of snake venoms and their antisera is due largely to the researches of Flexner and Noguchi [2] and of Kyes and Sachs.[3] The venoms of different snakes vary

[1] Calmette, Annal. Inst. Pasteur, Vol. vi, 1892; Comptes rend. Soc. Biol., 1894.

[2] Flexner and Noguchi, Journal Exp. Medicine, 1902, et seq.

[3] Kyes and Sachs. See in Collected Studies on Immunity, Ehrlich, New York, 1906.

a great deal in their toxic properties, and this is due to their relative contents of different constituents, as follows: — hæmagglutinins, hæmolysin, hæmorrhagin, and neurotoxin. The first two act exclusively on the blood cells, the hæmorrhagin on the endothelium of the blood vessels, and the neurotoxin on the cells of the central nervous system. The last named causes death by paralysis of the cardiac and respiratory centers. The venoms of the cobra, water-moccasin, daboia and some poisonous sea snakes are essentially neurotoxic, although they have strong dissolving powers for the erythrocytes of some animals. In studying the hæmolytic powers of the venoms of cobra, copperhead, and rattlesnake, Flexner and Noguchi found cobra venom to be the most hæmolytic and that of rattlesnake the least. They attribute the toxicity of rattlesnake poison chiefly to the action of hæmorrhagin. The venoms of the water moccasin and the copperhead also contain hæmorrhagin.

Unlike the bacterial toxins the action of the snake venoms is preceded by no appreciable incubation period. In addition to this the poisons are very rapidly absorbed. Thus Calmette found that a rat inoculated into the tip of the tail could not be saved by amputating the tail one minute later. Such animals died within about five minutes of the time required for control animals.

The hæmolysin and neurotoxin and perhaps also

the other cytotoxic substances of venom consist of amboceptors which find a complement in the body of the poisoned animal. Not only does ordinary serum-complement serve for activation, but, according to Noguchi,[1] the fatty acids contained in the red blood cells also act as complement.

Antivenins. — Calmette was the first to produce an antiserum against snake venom, utilizing for this purpose rabbits. He began with injections of $\frac{1}{10}$ of a fatal dose, and injected gradually increasing doses until at the end of four or five weeks the animals tolerated double a fatal dose. By continuing the treatment he finally got the animals to stand 80 fatal doses (40 mg.) without any reaction whatever. Five drops of the serum of such an animal neutralized 1 mg. cobra poison. It has been found that anticobra serum protects against the neurotoxic components of other snake venoms, furthermore against scorpion poison and the poison of eel blood. The serum also contains an antihæmolysin, but no antibody against hæmorrhagin (of the rattlesnake). It is therefore without effect on rattlesnake venom. Antivenin for the latter may be prepared by immunizing goats with corresponding venoms which have been attenuated by weak acids. Such a serum, of course, possesses no antineurotoxin and is therefore useless against cobra and viper venoms.

[1] Noguchi, Journ. Exper. Medicine, Vol. ix, 1907.

VIII. SERUM SICKNESS

Definition. — Under this name we now include the various clinical manifestations following the injection of horse serum into man. The principal symptoms of this disease are a period of incubation varying from eight to thirteen days, fever, skin eruptions, swelling of the lymph glands, leukonemia, joint symptoms, oedema and albuminuria. The term "serum sickness" was first used by von Pirquet and Schick,[1] from whose excellent monograph the following data are chiefly taken.

In 1874 Dallera reported that urticarial eruptions may follow the transfusion of blood. Neudörfer and also Landois also refer to this complication. In the year 1894 the use of diphtheria antitoxin introduced the widespread practice of injecting horse serum. In the same year several cases were reported in which these injections were followed by various skin manifestations, mostly of an urticarial character. Following these came a great mass of evidence which made it clear that following the injection of antidiphtheric serum these sequelæ were usually comparatively harmless. Nevertheless from time to time the occurrence of serious symptoms,

[1] v. Pirquet and Schick, Die Serum Krankheit, Wien, 1905.

and even of death, have been reported following the injection of diphtheria antitoxic serum. Rosenau and Anderson have collected nineteen such sudden death cases from the literature, and state they have personal knowledge of several more which have not been reported. However, considering the enormous number of antitoxic injections made each year, such accidents must be extremely rare. Certainly the benefits derived from diphtheria antitoxin far outweigh the danger. In over 50,000 persons injected in New York, but two deaths attributed to the serum furnished by the Health Department, have occurred.

Due to Serum as Such. — Heubner in 1894 and von Bokay somewhat later expressed the opinion that these manifestations were due to other properties than the antitoxin in the serum, and this has proven to be the case. Johannessen produced the same effects by injecting normal horse serum. It has also been shown that the skin eruptions and other symptoms follow in direct proportion to the amount of serum injected, and this has led to attempts to concentrate the serum as much as possible.[1] Park has also shown that the individuality of the horse plays an important rôle, some horses yielding a serum which gives rise to a large proportion of " rashes."

[1] See Gibson, The Concentration of Diphtheria Antitoxin, Jour. of Biological Chemistry, Vol. i, 1906.

Von Pirquet and Schick's Theory. — It was difficult to account for the long incubation period in "serum sickness." With poisons capable of self-multiplication (bacteria, etc.) this period was usually referred to the time necessary for them to accumulate in sufficient number and virulence to produce symptoms. But serum is not a poison capable of multiplication. Pfeiffer's work on the endotoxins led to the view that the antibodies played an important part in bringing on the symptoms by setting free the endotoxins. The results of these observations are very closely related to von Pirquet and Schick's explanation of the production of serum disease. The endotoxic theory, in the sense of bacteriolysis, naturally cannot be applied to albuminous substances in solution. We can only accept it in the sense that by means of the reaction between the antibodies and the antigen the poisonous substance is formed.

It is of course at once apparent that the formation of antibodies requires a definite period of time. The general idea underlying von Pirquet and Schick's theory of serum sickness is that the injection of the horse serum into man causes the development of specific reaction products which are able to act upon the antigens introduced. These antibodies encounter the antigens, i.e., some of the serum still present in the body, and so give rise to a poisonous substance. This accounts also for the cases of

"immediate reaction" described by von Pirquet and Schick, in which second injection of a serum produces an attack of serum sickness without any period of incubation. This includes also some of the cases of sudden death following the injection of horse serum. Here the second injection comes at a time when the accumulation of antibodies is at its height. Similar results were obtained independently by Rosenau and Anderson,[1] who found in the case of guinea pigs, that horse serum is poisonous to such animals as have been previously injected with small amounts of horse serum.[2] The time necessary to elapse between the first and second injections is about ten days. The symptoms are respiratory embarrassment, paralysis and convulsions, and come on usually within ten minutes after the injection. When death results it usually occurs within one hour, frequently in less than thirty minutes. The poisonous principle in horse serum appears to act on the respiratory centers. The heart continues to beat long after respiration ceases.

The first injection of horse serum renders the guinea pig susceptible; the quantity required for this purpose is extremely small. Rosenau and Anderson find that from $\frac{1}{250}$ to $\frac{1}{1000}$ c.c. ordinarily suffice. One tenth c.c. of horse serum injected into

[1] Rosenau and Anderson, Bulletin 29, Hygienic Laboratory, Washington, 1906.

[2] The Germans usually speak of this as "Theobald Smith's phenomenon of hypersusceptibility."

the peritoneal cavity of a susceptible guinea pig is sufficient to cause death. The same quantity inoculated substaneously may cause serious symptoms. Guinea pigs may be sensitized to the toxic action of horse serum by feeding them with horse serum or horse meat.

It may be that man cannot be sensitized in the same way that guinea pigs can. However, children have, in numerous instances, been injected with antidiphtheric horse serum at short and long intervals without, so far as we are aware, causing death. Certain serums, for example, the antitubercle serum of Maragliano and the antirheumatic serum of Mezer, are habitually used by giving injections at intervals of days or weeks. The results of Rosenau and Anderson make it probable that man may be rendered sensitive to the injection of a strange proteid, as is the case with the guinea pig and other animals, and that this explanation must be considered as well as the status lymphaticus, which has heretofore been assigned as the cause of sudden death following the injection of horse serum.

Anaphylaxis. — After the manuscript of the present volume had been sent to the printer, a splendid article on the subject of sudden death in "sensitized guinea pigs" made its appearance. The authors, Gay and Southard,[1] have adopted the term "ana-

[1] Gay and Southard, Journ. Medical Research, No. 98, May, 1907.

phylaxis " for the phenomenon. Their experiments indicate that the theory advanced by v. Pirquet and Schick is untenable, and they conclude that " the horse serum contains a substance, anaphylactin, which is not absorbed by the guinea pig tissue, is not neutralized, and is eliminated from the animal body with great slowness. When a normal guinea pig is injected with a small amount of horse serum, the greater part of its elements are rapidly eliminated ; the anaphylactin, however, remains and acts as a constant irritant to the body cells, so that their avidity for the other assimilable elements of horse serum which have accompanied the anaphylactin, becomes enormously increased. At the end of two weeks of constant stimulation on the part of the anaphylactin, and of constantly increasing avidity on the part of the somatic cells, a condition has arrived when the cells, if suddenly presented with a large amount of horse serum, are overwhelmed in the exercise of their assimilating functions, and functional equilibrium is so disturbed that local or general death may follow." The intoxication caused by the second injection depends upon constituents of the serum eliminable by the animal body.

According to Gay and Southard the tissue of the guinea pig examined during the anaphylactic phase showed no characteristic lesions. Striking multiple hæmorrhages accompany the toxic phase. The

hæmorrhages are more frequent in the stomach, cæcum, lungs, and heart than elsewhere.

It was natural to think that a formation of precipitins was in some way responsible for the symptoms of serum sickness or for the rare cases of sudden death following injections of antitoxin sera. It was conclusively shown, however, by v. Pirquet and Schick, Rosenau and Anderson, as well as others, that this is not the case. It was found, for instance that the symptoms of serum sickness appear within eight to thirteen days following the first injection of horse serum, whereas it requires about three weeks for precipitins to appear in the blood in children after the injection of horse serum. Furthermore, the formation of precipitins does not take place as readily in man following the injection of horse serum as it does in rabbits. In fact, v. Pirquet and Schick found that sometimes even after the injection of 200 c.c. there was no production of precipitins. Finally Rostoski has called attention to the fact that the precipitin action is a test-tube phenomenon only, and does not occur in vivo. It is well to bear these facts in mind. In a recent discussion on the treatment of severe cases of diphtheria in which the intravenous administration of large doses of antitoxin was recommended, one of the speakers alluded to the dangers from precipitin formation as contra indicating such a procedure. Such fears are groundless.

The Concentration and Purification of Antitoxic Sera. — Since the development of serum rashes and other disagreeable symptoms is largely associated with the serum as serum, it was natural that attempts should be made to concentrate the serum as much as possible. This was sought to be accomplished in two ways, — (1) by causing the production by the horses of a high grade serum, (2) by separating the non-antitoxic from the antitoxic fractions of the serum. Without going into details, we may say that the average grade of antitoxin at present produced is from five to ten times stronger than the early Behring sera. We have, however, in that time also markedly increased the number of units ordinarily given per dose, so that the volume of serum is still considerable. So far as the separation of the antitoxic and non-antitoxic fraction is concerned we have already referred to the great advance made by Gibson [1] in the practical concentration and purification of diphtheria antitoxin. It remains here to consider what clinical results have been achieved with this globulin preparation. In a recent study of this question Park and Throne [2] conclude that "the removal of a considerable portion of the non-antitoxic globulins, as well as all the albumins from the serum by the Gibson method has eliminated much of the deleterious

[1] See page 18.

[2] Park and Throne, Am. Journ. of the Med. Sciences, 1906.

matter from the serum, so that severe rashes, joint complications, fever, and other constitutional disturbances are less likely to occur from the antitoxic globulins than from the antitoxic serum from which it was obtained." Similar favorable reports have been published by other observers.

APPENDIX A.

THE WASSERMANN TEST FOR SYPHILIS.

As has already been pointed out on page 69, Wassermann applied the principle of the Bordet-Gengou phenomenon to the detection of syphilis antibodies in the serum and cerebrospinal fluid of persons infected with syphilis. In the two years which have elapsed since Wassermann's first publication, the reliability of this method of diagnosing syphilis has been confirmed by a large number of investigators, and it has already proven of considerable value in several departments of medicine. In response to numerous requests, the writer has undertaken to give a clear description of the test, together with a brief review of the results thus far achieved by its use.

When an animal is repeatedly injected with red blood cells of another species, it reacts to such injections by producing substances in its serum which have the power to dissolve these foreign blood cells. Examined by means of a test tube experiment, it is found that the serum exerts this power only while it is fresh. Serum several days old is unable to dissolve the red cells. The fresh serum also

loses its solvent power by exposure to heat, say to 55° C. Investigations showed that the solvent action could be restored to these sera by the addition of small quantities of a fresh normal serum, i.e. of a serum which by itself had no solvent power whatever. The inactive serum had thus been reactivated. The original specific dissolving serum therefore contained two substances, one of which is very labile and the other stable. The stable substance is specific for the blood cells against which it is directed, i.e. against the cells used for immunizing the animal. It is called the "immune body," or the "amboceptor." The labile substance, as we have seen, is present in all *fresh* sera, and is spoken of as the "complement." The action of the immune body seems to consist in bringing the solvent action of the complement to bear on the given cells. We must conceive that the complement possesses the solvent power, but has no way of laying hold of the cell to be dissolved. The immune body merely effects this combination. Ehrlich's diagram on page 64 will serve to make this conception clear.

All that has been said regarding immune bodies and complement for the solution of blood cells, holds for the substances which effect destruction of bacteria when bacteria are used for immunization. In fact, the process is the same, no matter what cells are injected into the animal. The immune body is always directed specifically against the cells injected, and against no others.

As can be seen from Ehrlich's diagrams, the bacteria or blood cells combine directly only with the immune body. The complement, as already said, has no way of laying hold of the cells. As soon as the bacteria or cells have anchored the immune body, however, conditions change. The combination at once attracts and unites with the complement. If the amount of complement is not too large, the combination may unite with all of it, i.e. may abstract the complement from the serum.

Just let us examine this by means of an illustration: Let us suppose we have immunized an animal with typhoid bacilli, and have obtained a specific serum directed against these bacilli. This serum has been inactivated by heating it to 55° C., so that now it will act on typhoid bacilli only when some fresh normal serum is added to complement the immune body. For this purpose we have provided ourselves with some freshly drawn serum from a guinea pig. The guinea pig serum, therefore, is the "complement." On mixing typhoid bacilli with the specific immune serum and then with the complement, these three factors enter into combination, and this results in the destruction of the typhoid bacilli. The quantities can easily be so arranged that this combination uses up all of the complement, so that the fluid contains not a trace of free complement after the substances have combined.

Suppose, now, that we also had a specific serum obtained by injecting an animal with red blood

cells, for example, by injecting a rabbit with sheep blood cells. This rabbit serum would then be specifically directed against sheep blood cells. Let us inactivate this serum, by heating it to 55° C., so that now it requires the addition of a fresh normal serum to exert its solvent effect. For this purpose we can again use fresh, normal guinea-pig serum. When, then, we mix sheep blood cells with our specific immune serum (against sheep blood cells) and with the complement, i.e., with fresh normal guinea-pig serum, all three factors unite, and bring about destruction of the red blood cells. This is manifested by the blood cells dissolving and giving off their hæmoglobin to the rest of the fluid.

Let us now suppose we have carried out the first part of this experiment, that with the typhoid bacilli, and have left typhoid bacilli, specific typhoid serum and complement in contact for several hours in a warm place in order to cause the three factors to combine. At the end of this time let us add sheep blood cells and the specific serum directed against sheep cells, but let us add no further complement, because the fresh guinea-pig serum was able, as we saw, to serve as as complement also for the blood combination. The mixture is again placed in a warm place for several hours, and then for twenty-four hours in the refrigerator, after which it is examined. We shall find that no hæmolysis has

-red, from which we conclude that the previous

nation (typhoid bacilli, immune serum and

complement), had used up all the complement, and left none for the blood combination.

If we were to repeat the whole experiment, but leave out, in the first part of the test, say the specific typhoid serum, we should find that the blood cells would be dissolved. This is readily understood when it is remembered that then we would have only typhoid bacilli and complement, two factors which cannot combine directly. The complement would therefore be left free to act in the blood combination.

If hæmolysis occurs we may therefore conclude that one of the factors in the first combination was absent, and conversely, if hæmolysis does not occur, we know that the first combination must have been perfect, i.e. all three factors must have been present.

It is at once apparent that in adapting this test to the detection of syphilis antibodies, pure cultures of the causative organism, i.e. of the "antigen," were not available. Wassermann therefore made use of extracts of syphilitic organs rich in spirochætes in place of the typhoid bacilli, and used either the serum or the cerebrospinal fluid of the suspected case in place of the typhoid antiserum. The rest of the test was similar to that described above. When hæmolysis of the sheep cells occured, Wassermann said it showed that the first combination was incomplete; when hæmolysis was completely inhibited, it showed, he said, that the first combination was perfect, i.e. that the serum or

spinal fluid contained syphilis antibody. As in most such tests, only a positive result determines; a negative result does not necessarily exclude the presence of syphilitic infection.

While the above exposition will serve to fix the general plan of the test in the mind of the reader, we must at once say that the mode of action is not as simple as Wassermann first believed. Before going into this phase of the subject, it will be advisable to present a description of the technique of the test.

For carrying out the test the following materials are required:

(1) Antigen, i.e. fluid containing syphilis material. This is comparable to the pure culture of typhoid in the test described above.

(2) Serum or cerebrospinal fluid from the patient to be examined.

(3) Sheep blood cells.

(4) Hæmolytic antibody, i.e. inactivated serum of a rabbit immunized against sheep blood cells.

(5) Complement, i.e. fresh normal serum from a guinea-pig.

For the syphilis antigen it is best to use the organs of a syphilitic fœtus, i.e. one dead of hereditary syphilis, as these tissues are particularly rich in spirochætes. The organs are chopped up and macerated in a clean vessel in a mixture composed of water, 1000; NaCl, 8.5; carbolic acid, 5.0; one part of the tissue to four of the fluid. The mixture is shaken in a shaking apparatus for twenty hours;

the supernatant fluid poured off and centrifuged so as to be perfectly clear.*

The serum for the test is collected from the patient in the usual way by drawing from 5 to 10 cc. of blood from a vein at the elbow, placing the blood in a sterile test tube and allowing it to clot. Cerebrospinal fluid, obtained by lumbar puncture, is preserved with 0.5% carbolic acid, and then strongly centrifuged so as to make it perfectly clear.

The sheep blood cells are obtained by defibrinating sheep blood, centrifuging and washing the blood cells repeatedly with normal salt solution to remove traces of adherent serum. A 5% suspension in salt solution is used.

The hæmolytic antibody consists of the serum of a highly immunized (against sheep blood cells) rabbit, the serum being inactivated by heating to 56° C. In the tests cited by Wassermann, one cc. of a 1/1500 dilution of serum dissolved one cc. of 5% suspension of sheep blood cells at 37° C. in two hours.

The complement consists of freshly drawn guinea-pig serum. The test is carried out as follows:

To constant quantities of spinal fluid (e.g. 1 cc. of the 1/10 dilution) decreasing amounts of the extract of organs are added, thus 0.2, 0.1, 0.05 cc. Then 1 cc. of a 1/10 dilution of fresh normal guinea-

* In a very recent article, Wassermann states that more uniformly active extracts can be obtained by using 96% alcohol in place of the water in the above formula.

pig serum is added, and the mixtures allowed to remain in contact at 37° C. for one hour in order to bind the complement.

In this mixture we have *antigen;* we may or may not have *antibody;* we have *complement.*

If the antibody is present, the complement will be anchored by the combination, and so be unavailable for the hæmolytic test next in order. If no antibody is present, the complement will still be free to act in the hæmolytic test.

At the end of the hour, we add to the above mixtures: one cc. of a 5% suspension of sheep blood cells, and one cc. of the amboceptor dilution containing double the solvent dose for that amount of sheep blood cells. Thus, if the titer of the hæmolytic serum is 1/1800, we take one cc. of a dilution 1/900.

All the tubes are made up to the same volume with normal salt solution, namely, to 5 cc., and are then placed in the incubator at 37° C. and kept there for two hours. Then they are placed on ice until the next day, when the results are noted. The whole procedure is clearly shown by the protocol from Wassermann and Plaut reproduced on page 155.

Few experiments in immunity require such careful technique, or are open to so many sources of error as this serum test for syphilis. In view, too, of the enormous responsibility assumed in making a positive diagnosis of syphilis, it is apparent that the test should only be undertaken by

Syphilit. Fœtus Extract. (0.2 gm.) One c.c. of $\frac{1}{5}$ Dilution.	Spinal Fluid of Patient M. 0.2, i.e., One c.c. of the $\frac{1}{5}$ Dilution.	Normal Guinea-pig Serum 0.1 cc., i.e. 1 cc. of a $\frac{1}{10}$ Dilution.	Hæmolytic Amboceptor 1 cc. equals Double the Solvent dose for 1 cc. of a 5% Suspension.	Sheep Blood Cells 1 cc. of a 5% Suspension.	Results.
0.2	0.2	1.0	1.0	1.0	Complete inhibition of hæmolysis
0.1	0.2	1.0	1.0	1.0	Compl. inhibition
0.2	0.1	1.0	1.0	1.0	Marked inhibition
0.1	0.1	1.0	1.0	1.0	Marked inhibition
0.2	—	1.0	1.0	1.0	Complete solution
0.1	—	1.0	1.0	1.0	Complete solution
—	0.2	1.0	1.0	1.0	Complete solution
—	0.1	1.0	1.0	1.0	Complete solution
	Spinal Fluid of Non-syphil. Person.				
0.2	0.2	1.0	1.0	1.0	Complete solution
0.1	0.2	1.0	1.0	1.0	Complete solution
0.2	0.1	1.0	1.0	1.0	Complete solution
0.1	0.1	1.0	1.0	1.0	Complete solution
—	0.2	1.0	1.0	1.0	Complete solution
—	0.1	1.0	1.0	1.0	Complete solution

highly trained laboratory workers. On the other hand, most who have busied themselves with the test agree that suitable controls always lead to a detection of possible sources of error, and that

therefore the reaction, when properly performed, can be relied upon.

When the test was first published Wassermann regarded the reaction which occurred as one between mutually specific bodies, i.e. between antigen and antibody, the resulting combination having the power to anchor the complement. Through the work of Marie & Levaditi, of Landsteiner, Müller and Pötzl, of Weil and Braun, and still other investigators, it soon became apparent that the test could also be carried out by using extracts of *non-syphilitic* tissue, i.e. of other pathological tissue or normal tissue. That, of course, meant that the view of a reciprocal specific relation between antibody and organ extract, in the sense that typhoid antibody and typhoid bacilli are reciprocally related, had to be abandoned. This does not, however, effect the reliability of the reaction for diagnostic purposes, for it has been found that positive results are still only obtained when the serum or spinal fluid is of syphilitic origin.*

Working under Wassermann's direction, Porges and Meier studied the nature of the substances concerned in the reaction, and began by precipitating the organ extracts with alcohol and testing

*It may be well to state that according to Landsteiner, Müller and Pötzl the serum of animals infected with dourine (trypanosomiasis) also gives rise to inhibition of hæmolysis when tested according to the above method. This has been confirmed by Hartoch and Yakimoff. Whether this will affect the value of the Wassermann test in humans can only be decided by further clinical tests, especially in cases of human trypanosomiasis.

the resulting precipitate and clear fluid separately. It was found that the substance concerned in the reaction was soluble in alcohol, and the authors thereupon made alcoholic extracts of the syphilitic organs. These worked satisfactorily in making the test. It was natural to think that the substance which effected the reaction might be related to the lipoids, and so the authors next studied the behavior of alcoholic extracts of *normal* human and animal organs. While these extracts also sufficed to produce the reaction, it was evident that they were not as active as extracts from syphilitic organs. After it had been found that alcoholic extracts could be used for the test, a number of authors almost simultaneously published favorable results with chemically defined substances. Porges and Meier used lecithin, Levaditi glycocholate of soda, Sachs and Altmann oleate of soda, and Fleischmann even used vaseline. The last-named also used cholesterin with favorable results, but Porges and Meier obtained only negative results with this substance. On the whole, however, it seems that the extracts, especially of syphilitic organs, give the most uniform results.

At the present time, therefore, Wassermann believes that the really active principle in the antigen may be a combination of lipoids with certain protein-like substances, and that the latter component, when it is derived from syphilitic material, has something of a specific character. In this connection Wassermann refers to the researches of

Noguchi, Landsteiner, and others which show that minute quantities of proteid mixed with lipoids may cause extensive alterations in the physico-chemical behavior of the latter. He thinks that under certain circumstances this proteid component may play an important role in determining the reliability of the reaction, a view which is borne out by the investigations of Neisser and Bruck.

While Porges and Meier were engaged in the studies just mentioned, Fornet and also Michaelis showed that when the serum of individuals infected with syphilis was mixed with certain antigens a zone of precipitation might at times be observed at the point of contact of the two fluids. The antigen employed by Fornet was serum from individuals in the florid stage of syphilis; Michaelis used extracts of organs from a syphilitic fœtus. This of course agrees with what was already known from the work of Bordet, Gengou, and Gay. In fact, according to Gay, the deflection or absorption of complement, on which the Bordet-Gengou test depends, may be due to the precipitate formed in the combination. While this is true, complement may also be anchored in the Bordet-Gengou test without the formation of any precipitate.

Porges and Meier thereupon tested the alcoholic extracts, and solutions of lecithin and of glycocholate of soda to see whether this zone of precipitation was at all constant, and whether it might not be possible to substitute such a simple precipitation test for the complicated Wassermann reaction.

While it was found that the test was roughly specific, it was soon realized that a precipitate might at times be produced with the serum of surely non-syphilitic individuals, and similar unfavorable results have since been published by other authors. At the present time, therefore, the only reliable serum diagnosis of syphilis is that based on the absorption of complement.

The results obtained with the Wassermann test are well reflected in the findings of Fleischmann, as follows:

The total number of persons tested was 230, of which 38 were controls. None of the latter gave a positive reaction. The other cases can be arranged into four groups thus:

(1) Cases surely syphilitic, with clinically manifest signs of syphilis at the time of the test. Of 89 such cases tested, 83 gave a positive reaction (93%).

(2) Cases surely syphilitic but without clinical symptoms at the time of the test. Of 64 such cases tested, 33 gave a positive reaction (52%), and 31 gave a negative reaction (48%).

(3) Cases with symptoms suggestive of syphilis, and with an indefinite history of infection. Of 32 such cases, 16 gave a positive reaction (50%), and the rest a negative reaction.

(4) Surely syphilitic individuals showing cutaneous lesions which the dermatologists diagnosed as very probably not syphilitic. Of 7 such cases, 1 gave a positive reaction and the rest a negative reaction.

Bruck and Stern tested 378 cases suspected to be

syphilitic, and obtained a positive reaction in 204. They also tested 157 surely non-syphilitic individuals as controls, and found all but two negative. These two gave a doubtful reaction.

In a recent paper Wassermann has collected data on about 3000 tests, as follows: There were 1010 tests on cases surely non-syphilitic (controls), and not one of these gave a positive reaction. Of the 1982 surely syphilitic cases tested, those examined at the time when they had manifest symptoms reacted in about 90% of the cases. When the cases tested were without manifest symptoms, so-called "latent syphilitics," about 50% reacted.

As a matter of interest it may be mentioned that Blumenthal and Wile tested the urine of syphilitic individuals, and found that this too would give the reaction.

Marie and Levaditi examined the *cerebrospinal fluid* of 30 cases of general paresis. All but two of the cases gave a positive reaction. When the *serum* was tested in place of the cerebrospinal fluid, the percentage of positive findings dropped to 59%.

Michaelis examined 20 cases of general paresis and obtained a positive reaction in 18 of them.

Citron examined 43 tabetics and paretics, and obtained a positive reaction in 34 cases (79%). He also tested the serum of 108 persons surely infected with syphilis, or suspected to be infected, and obtained a positive reaction in 80 (74%). None of the sera from 156 surely non-syphilitic individuals gave a positive reaction.

Favorable reports have also been published concerning the reliability of the test in ophthalmology, dermatology, and other departments of medicine.

At the present time we may therefore say that the chief value of the serum test for syphilis will be in those cases in which there are symptoms suggestive of syphilis and in which the history of the case fails us or is of questionable reliability. In such instances a positive reaction at once clears the diagnosis, and sometimes even a negative reaction is helpful.

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APPENDIX B.

NOGUCHI'S BUTYRIC ACID TEST.

FEELING that the lecithin, glycocholate of soda, oleate of soda, and other compounds which had been used in the various modifications of the Wassermann test, might act as acids, and that this acidification possibly gave rise to a precipitate of globulins and related substances, Noguchi sought to discover the nature of the Wassermann reaction by studying the influence of various acids on the serum of different individuals. He found that in general a greater degree of precipitation was produced in syphilitic sera than in non-syphilitic ones. It was natural to think that the deflection of complement observed in the Wassermann test was due to adsorption by the precipitate as such, but on testing the precipitate itself this was found not to be the case.

The increased precipitation produced in syphilitic sera by the addition of acid suggested an increase and qualitative change on the part of the serum globulin, and this possibility was also indicated by the results obtained by Klausner. This author, it may be stated, devised a test for syphilis, based on the formation of a precipitate when the serum

was mixed with distilled water. Noguchi then made exact determinations of the globulin content of the different sera, and found that in cases of secondary syphilis either untreated or but slightly treated, an increased globulin content could be demonstrated. In primary and tertiary stages the change was found to be inconstant. The results of these globulin determinations were quite generally paralleled by the result of the Wassermann test on the sera question. The method employed for determining the globulin content of the serum was the ordinary one of the biochemical laboratory, as follows:

The serum was mixed with half-saturated solution of ammonium sulphate and the resulting precipitate concentrated always to the same volume by means of a centrifuge. After pouring off the supernatant fluid, the precipitate was carefully weighed in its moist condition.

It is evident that this method can be employed only by trained workers in suitable laboratories: it is hardly clinically applicable. In order to overcome this objection, Noguchi devised the following simple modification:

The ammonium sulphate precipitate is separated by contrifuging as before, and the supernatant fluid poured off. The precipitate is then redissolved by adding ten volumes of physiological salt solution, and tested by the addition of a 10% solution of butyric acid in salt solution. When the globulin content of the serum is normal slight

opalescence is produced, but with an increased globulin content, such as is seen in secondary syphilis, the mixture becomes cloudy and shows a distinct flocculent precipitate in about half an hour. With butyric acid the appearance of the precipitate is quite characteristic; with other acids much less differentation is obtained.

The technique of the butyric acid test is as follows:

For Serum—To 1 cc. serum add 4 cc. half-saturated solution ammonium sulphate. After two hours, centrifuge at high speed for 15 minutes. Pour off the supernatant fluid, and dissolve the precipitate in 10 cc. physiological salt solution. To 0.5 cc. of this solution add 0.5 cc. of a 10% solution of butyric acid in salt solution. A flocculent precipitate within two hours constitutes a positive test. Readings made after that time may lead to erroneous conclusions, as even non-syphilitic sera may give a slight precipitation under these circumstances.

For Cerebrospinal Fluid.—The preliminary precipitation with ammonium sulphate is omitted. To 0.1 cc. of the fluid add 0.5 cc. of the 10% solution of butyric acid in salt solution. Heat to boiling, and add 0.1 cc. normal NaOH. Observe the tubes at the end of ten to twenty minutes. A positive reaction is indicated by the appearance of a coarsely granular or flocculent precipitate. With a negative reaction there is merely a uniform clouding, but no such precipitate.

Noguchi's results with the butyric acid test are shown in the following summary:

(1) The cerebrospinal fluid of 40 cases of general paresis was tested. 40 gave a positive reaction with Noguchi's test; 34 a positive reaction with Wassermann's test.

In all the cases where a positive reaction was obtained, the diagnosis agreed with that based on the cytological and clinical findings.

(2) The cerebrospinal fluid of 43 individuals, comprising cases of alcoholic psychoses, dementia præcox, epileptic and other cerebral affections, was tested. None of these gave a positive reaction with Noguchi's test; two gave a faint reaction with Wassermann's test.

(3) Tests made on the serum of syphilitic individuals gave the following results:

Stage of Infection.	Wassermann Test.		Noguchi Test.	
	Positive.	Negative.	Positive.	Negative.
Primary syphilis:				
Untreated	3	1	4	0
Treated	0	1	0	1
Secondary syphilis:				
Untreated and little treated	13	1	14	0
Well treated or still under treatment	1	11	5	7
Tertiary syphilis	1	2	1	2

(4) The serum of 17 normal, non-syphilitic individuals was tested, without a positive reaction with either test. It may, however, be stated that a

positive reaction was obtained in an advanced case of tuberculosis, but it was not possible to say definitely that a syphilitic infection was not also present.

According to Noguchi, the Wassermann test occasionally gives negative results in general paresis; the butyric acid test always gives a positive reaction, giving results which agree perfectly with those obtained by cytodiagnosis, and with the clinical picture. Noguchi's test for spinal fluid can be applied even to old specimens (a year old) with equally good results as in fresh fluid. Wassermann's test requires fresh spinal fluid. Whether this simple test can be substituted for the well-tried Wassermann reaction can only be determined by a large number of careful control experiments.

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